

3D PRINTING IN BIOTECHNOLOGY

Current Technologies and Applications

Nandita Dasgupta, Vineeta Singh,
Shivendu Ranjan, Taijshee Mishra, and Bhartendu Nath Mishra



3D Printing in Biotechnology

Additive Manufacturing Materials and
Technologies

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Current Technologies and Applications

Nandita Dasgupta

International Research Center in Critical Raw Materials for Advanced
Industrial Technologies (ICCRAM), Universidad de Burgos, Burgos, Spain

Vineeta Singh

Department of Biotechnology, Institute of Engineering and Technology,
Lucknow, Uttar Pradesh, India

Shivendu Ranjan

School of Nano Science and Technology (SNST), Indian Institute of
Technology Kharagpur, Kharagpur, West Bengal, India

Taijshee Mishra

NITI Aayog, New Delhi, India

Bhartendu Nath Mishra

Department of Biotechnology, Institute of Engineering and Technology,
Lucknow, Uttar Pradesh, India

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Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
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Preface

3D printing or additive manufacturing is currently being explored for various applications and industrial uses. It has the potential to transform the design and manufacturing processes with customizable changes in process and product design. This redesign capability can be used for increasing material or energy efficiency, safety, and sustainability for the entire product lifecycle. 3D printing has opened new possibilities and dimensions in the field of biotechnology. The ability to recapitulate biological systems and processes with high precision and reduced risk of inflammatory reaction can be applied in various research areas, including drug delivery, tissue engineering, and food and waste management. Figure 1 highlights the role of 3D printing in tissue engineering, wherein it is possible to mimic the functions of human liver.

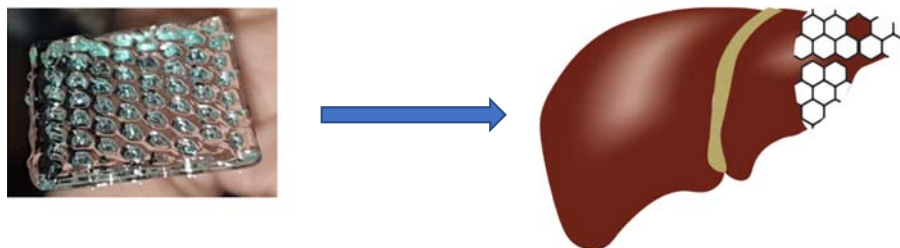


Figure 1 Possibility of 3D-printed scaffolds to mimic the structural and functional aspects of human organs.

The book provides a snapshot of the recent ongoing research on how 3D printing can be used in biotechnology. It first gives an overview of the various currently used techniques or types of 3D printers being used for different end applications specific to biotechnology, such as for biomedical devices, diagnostics, therapeutics, tissue implants, scaffolds, organoids, and others mentioned in Chapter 1. Chapter 2 deals with different types of materials, polymers, or bionks used in 3D printing of scaffolds, while Chapter 3 provides an in-depth discussion of 3D-printed tissue scaffolds, their application, limitations, and future scope. Chapter 4 deals specifically with 3D-printed grafts and metal implants while Chapter 5 deals with personalized drug delivery approaches with 3D printing. Chapters 6 and 7 explore 3D printing applications in food and waste management, respectively. The authors provide information about the recent emerging trends and techniques in 3D printing in Chapter 8. The book also covers the sustainability, ethical, and regulatory issues in

Chapters 9 and 10. Throughout the book, the authors have demonstrated various examples and offered their insights into each area.

Overall, the book offers technical overview and insights that will be helpful for researchers and medical professionals who are working in various branches of biotechnology. The authors believe that the book will be helpful in contributing toward the widespread adoption of this new technology in various market-wide applications.

Thanks for reading.

Nandita Dasgupta
Vineeta Singh
Shivendu Ranjan
Taijshee Mishra
Bhartendu Nath Mishra

Three-dimensional printing in biotechnology: techniques and applications

1

Chapter outline

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Introduction

Three-dimensional (3D) printing is a digitized and flexible technology that converts geometrical forms into physical objects by successive addition of materials. The first 3D printer based on Stereolithography (SLA) was developed by C. W. Hull in 1984. The 3D printing technology is also popularly known as additive manufacturing (AM) and nowadays widely used due to its distinctive advantages in various applications in aerospace, architecture, fashion, food, and biologics industries (Godoi, Prakash, & Bhandari, 2016; Jiang, Yu, Xu, Ma, & Liu, 2020; Liu, Hamid, Snyder, Wang, & Sun, 2016). The computer-aided design (CAD)/3D modeling or image processing software are used to design 3D structures and thereafter these designed objects are fabricated in a layer-wise manner using a 3D printer.

3D printing in biological and biomedical field is also termed as 3D bioprinting, which allows the development of blood vessels as well as customized tissues for

implantation as heart valves, trachea, and myocardial tissues. 3D-printed flowers, artificial photosynthetic systems, wearable electronics, e-plants, and cell-laden scaffolds are some of the applications of this novel technology in plant science research. Furthermore, 3D-printed fabricated ecosystems, EcoFABs, allow the controlled study of plant microbiome interaction (Zengler et al., 2019).

Evolution of three-dimensional Bioprinting

Klebe (1988) demonstrated the first application of bioprinting by depositing cells in 2D structure using a HP inkjet printer. Furthermore, Odde and Renn (1999) demonstrated the first 3D patterning of live cells to form complex tissue analogs using laser-assisted bioprinting. In 2002, Landers, Hübner, Schmelzeisen, and Mülhaupt (2002) developed the first extrusion-based bioprinter that was later commercialized as 3D-Bioplotter. In 2003, Wilson and Boland (2003) bioprinted live cells by modifying the office inkjet printer into inkjet-based bioprinter. Further developments in 3D printing took place to minimize the cost of printing, so as to make it affordable for every end user. In 2006, Jayasinghe, Qureshi, & Eagles (2006) used electric field-driven jetting phenomenon, known as electrohydrodynamic (EHD) jetting, to deposit living cells. In 2009, Norotte, Marga, Niklason, and Forgacs (2009) developed scaffold-free vascular tissue through bioprinting. In 2012, Skardal et al. (2012) made an attempt of bioprinting a mouse model. In 2015, Gao et al. (2015) developed coaxial technology for the fabrication of tubular structure. In 2019, Lee, Abelseth, de la Vega, and Willerth (2019) succeeded in bioprinting collagen to develop human heart model using suspended hydrogel technology. With the abovementioned examples, it is evident that 3D bioprinting techniques have the potential to address challenges of conventional tissue engineering fabrication methods. A brief timeline of historical developments in 3D printing/3D bioprinting as well as their future applications is given in Fig. 1.1.

3D bioprinting techniques offer selective distribution of cells, biomaterials, growth factors, or combinations thereof, to manufacture living tissues and organs in three dimensions. Fabrication of such cell-laden constructs, thus, helps in steering cellular activity. Biocompatible materials, such as polymers, hydrogels, and composite materials, are used to make scaffolds to mimic the complex architecture and mechanical properties of natural tissues. The development of solvent-free, aqueous-based systems enable direct printing of biological materials into 3D scaffolds that can be used for transplantation with or without seeded cells (Murphy & Atala, 2014). Today, various tissue models derived from 3D bioprinting techniques are being used to examine drug delivery pathways. Moreover, 3D bioprinting allows the development of novel drug delivery systems with unprecedented precision and complexity. Furthermore, it enables achieving detailed spatial composition and controlled release pattern of drug, which was not possible through previous techniques. Now, patient-specific, customizable, and on-demand personalized medicine, implants, and wearable devices are becoming a reality due to the speed and flexibility offered by 3D printing technology.

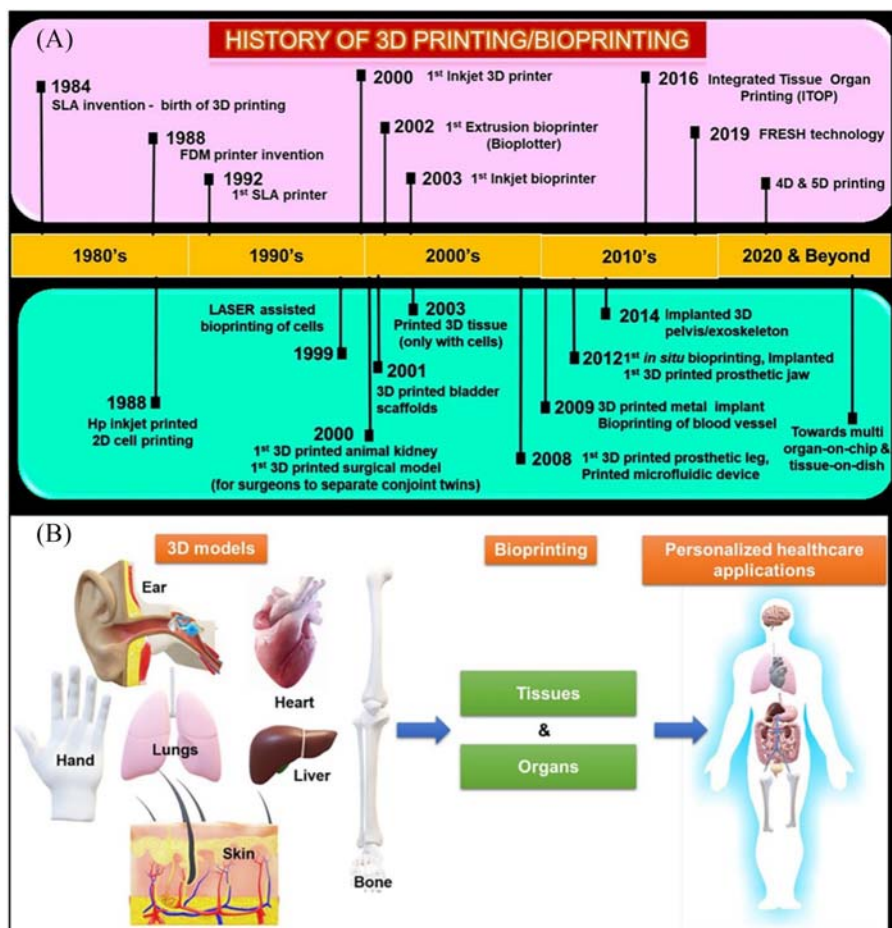


Figure 1.1 (A) A brief timeline of historical developments in 3D printing/bioprinting. (B) Futuristic application of bioprinting of tissues and organs for the development of personalized organs (Sekar et al., 2021).

Source: Reproduced with permission from Sekar, M.P., Budharaju, H., Zennifer, A., Sethuraman, S., Vermeulen, N., Sundaramurthi, D. & Kalaskar, D.M. (2021) Current standards and ethical landscape of engineered tissues-3D bioprinting perspective. *Journal of Tissue Engineering*, 12, 20417314211027677. <https://doi.org/10.1177/20417314211027677>.

The selection of biomaterials for bioink formulation depends on the printability of materials, its characteristics, and properties for external applications. Whereas bioink for implantable constructs require characteristics of biomaterials that are specific for both physiological conditions and interactions with the human body. Therefore, overall, printable biomaterials must have characteristics such as printability, biocompatibility, mechanical properties, degradation kinetics, and should

exhibit tissue mimicry. These properties are linked to the type of printing methods being used. For example, in bone tissue formation, stiff materials are required for load-bearing capacity. Due to the inherent stiffness of printed biomaterials, the majority of 3D-printed constructs are suitable to be used in bone or cartilage applications mimicking the natural stiffness of these tissues. However, it certainly makes discovery and development of novel biomaterials more challenging.

Three-dimensional bioprinting technology

The 3D printing process starts with the creation of a 3D model using CAD software or 3D scanning system (optical, MRI, CT, or laser). Once the 3D model is developed, it needs to be converted to the STL file format to be recognized by slicer software, which stores the information of the model surfaces as a list of coordinates of triangulated sections. The slicing process converts the stored information or data into a G-code file. This G-code file contains all geometrical information or data of the 2D cross-section of the designed object to be printed. The printer then avails G-code files to deposit the bioink material layer by layer, which is built one upon another until the desired 3D object is finally created. A working diagram of 3D printing process is given in Fig. 1.2. Once printing is finished, the bioprinted object is sent for postprocessing operations to finally obtain a finished bioconstruct.

In 3D bioprinting, the material being printed is called bioink, which consists of living cells, biomolecules, and biomaterials to actively print the tissues of living cells. The 3D bioprinting of tissue and organs finds many applications in regenerative medicine. For some bioprinting applications, the process workflow typically starts with data acquisition by MRI or CT of the tissue or organ to be fabricated. The medical image data sets, thus, provide essential information about the macrostructure of the tissues and organs. However, in case where the information at microstructure is not possible, the advanced microscopy techniques (fluorescent, confocal, or two-photon) can provide some crucial details at the cellular level. Currently, MRI or CT data sets are mainly used to design the overall volume of the object to be fabricated, while the information about the infill is normally designed through open source or proprietary softwares. Therefore, it is evident that there are still challenges that need to be addressed for a more innovative bioprinting

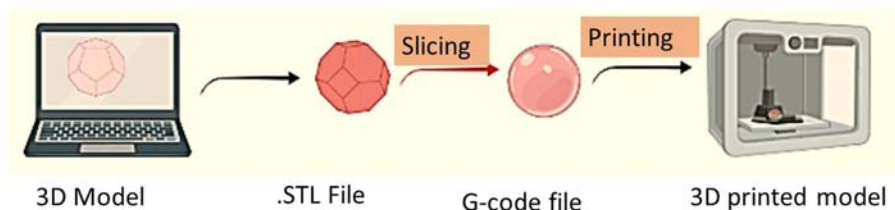


Figure 1.2 Representation of three-dimensional (3D) printing working diagram from designing of object to obtain 3D-printed model.

strategies. Thus, the true power of the 3D bioprinting technology is yet to be explored. It might take some more time to establish this technology as gold standard in biotechnology research.

Three-dimensional bioprinting techniques

Over the past decade, several bioprinting techniques have emerged based on different driving and dispensing mechanisms and, presently, these techniques are being used to fabricate various tissues and organs by selectively dispensing cells or hydrogels, or combinations thereof. 3D bioprinting techniques are broadly categorized into three major modalities, namely extrusion-based, light-assisted, and droplet-based, which are further subclassified into various categories according to material type, cell viability, and surface resolution. A broad classification of various 3D bioprinting techniques is given in Fig. 1.3. Each technique has its own unique advantages and constraints. Hence, selection of a suitable bioprinting technique is utmost important to obtain 3D bioprinted construct of choice. A comparison of various bioprinting techniques is given in Table 1.1.

Extrusion-based bioprinting

Extrusion-based bioprinting (EBB) techniques use pressure as a driving force for bioprinting. It consists of a print head, a print stage, and a control system for controlling printing speed, temperature, and print location. In this technique, a continuous strand of bioink is extruded through a syringe and needle by gravitational and/or mechanical force, or by pressurized air (Ning et al., 2020). EBB has fast printing speed and can deposit large cell densities, which makes it a viable technique for large-sized scaffolds. Depending on the driving force and dispensing mechanism, EBB can be classified into two types, i.e., mechanical and pneumatic. The former

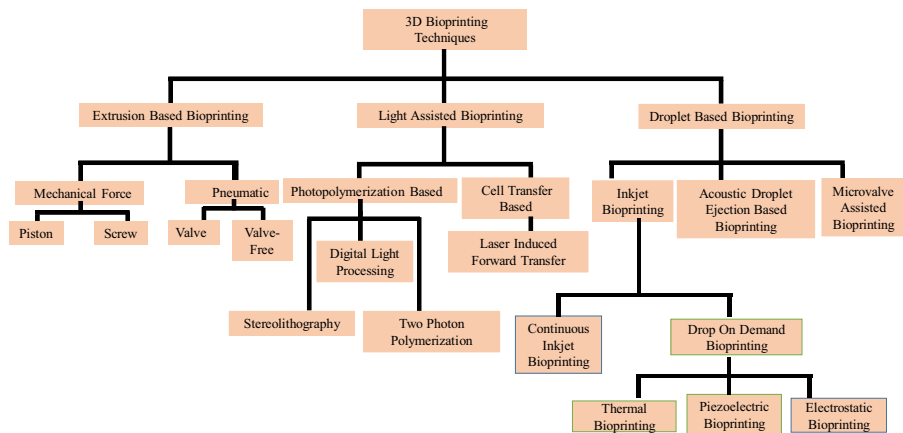


Figure 1.3 Classification of three-dimensional bioprinting techniques.

Table 1.1 Comparison of three-dimensional bioprinting techniques.

	3D bioprinting approaches			
	Extrusion based	Light based	Stereolithography	Droplet based
Actuation method	Pneumatic and mechanical	Light-induced pulse	UV laser and UV light-based curing	Thermal, piezoelectric and electrostatic
Gelation method	Photo-crosslinking	Chemical and photo-crosslinking	Photo-crosslinking	Chemical and photo-crosslinking
Cell viability	60%–90%	More than 90%	More than 85%	More than 85%
Cell density (cells/mL)	High	Medium	Medium	Low
Resolution	Moderate 100 μm wide	High 10 μm wide	High 50 μm wide	High 50 μm wide
Printing speed	Slow 10–50 $\mu\text{m/s}$	Medium 200–1600 mm/s	Fast	Fast 10,000 drops/s
Advantages	High cell density, User-friendly and capable of printing various biomaterials	Ability to print low viscosity biomaterials, fast fabrication speed, low cost, and high resolution	Nozzle-free technique, high print speed, high accuracy, and high cell viability and printing time is independent of complexity	Ability to print low viscosity biomaterials, fast fabrication, cost-effective and high resolution
Disadvantages	Shear stress causes cell damage Problem of needle clogging This technique is applicable only for viscous liquids	Suffers cell damage due to nanosecond/femtosecond laser High cost	Damage to cells while photocuring due to UV light toxicity Inability to print multicellular structure This technique is only applicable for photo-crosslinking bioinks	Difficulty to dispense viscous materials Inability to provide a continuous flow Clogging problem
References	Cui et al. (2020); Ramesh et al. (2021)	Antoshin et al. (2019); Wang et al. (2018)	Xu et al. (2020); Xu et al. (2021)	Graham et al. (2017); Gudapati et al. (2016)

utilizes mechanical force for extrusion through either piston or screw-driven system. Piston is used for the extrusion of cell-laden bioinks, whereas screw-driven system is used for the printing of acellular materials. Valve-based or valve-free pneumatic 3D bioprinters use air pressure to promote the extrusion of bioink through the print head and nozzle. Despite sharing the same working principle, the two platforms (mechanical and pneumatic) utilize different mechanisms for dispensing bioink. A schematic diagram of mechanical extrusion bioprinting and pneumatic extrusion bioprinting is given in Fig. 1.4.

The major limitation of EBB is that during bioprinting, the incorporated cells go through pressure and shear forces, which may lead to rupturing of cell membranes and further cause loss in their integrity if the process-induced forces exceed the cell membrane threshold. Moreover, extrusion printing has low resolution and experiences clogging problems (Naghieh, Sarker, Sharma, Barhoumi, & Chen, 2020). In spite of these limitations of EBB, due to its versatility, affordability, and ability to print porous constructs and large constructs, it has now been utilized by researchers worldwide to bioprint cells, tissues, tissue constructs, organ models, and organ-on-a-chip. Thus far, a wide variety of tissue constructs have been successfully fabricated with EBB, such as cartilage, vasculature, bone, skin, liver, and cardiac constructs, employing bioinks containing cells, tissue spheroids, decellularized matrix components, cell-laden hydrogels, and microcarriers.

The working principal of extrusion bioprinting is similar to fused deposition modeling (FDM, a 3D printing approach that uses melted filament materials as ink) and is widely used in the development of drug delivery systems, personalized



Figure 1.4 Schematic diagram of extrusion-based bioprinting (A), pneumatic extrusion printing (B), and (C) mechanical extrusion bioprinting (Jeong, Nam, Jang, and Lee, 2020). Source: Reprinted from Jeong, H.-J., Nam, H., Jang, J., Lee, S.-J. (2020). 3D bioprinting strategies for the regeneration of functional tubular tissues and organs. *Bioengineering*, 7, 32.

medicine, and biomedical devices. Polycaprolactone is a widely used biocompatible material for applications in wound dressings, tissue engineering, and drug delivery. Thermoplastic polyurethane is another biocompatible material broadly used for bone regeneration, bone replacement, and drug or gene delivery.

Light-assisted bioprinting

Light-assisted bioprinting (LAB) is an emerging and promising in situ bioprinting technique that offers high printing resolution and precision. This bioprinting system consists of a light source, a transparent substrate, a biocompatible material to be printed and an energy-absorbing layer. LAB can be broadly classified into two types, i.e., photopolymerization-based and cell transfer-based. Photopolymerization-based bioprinting techniques include stereolithography (SLA), digital light processing (DLP), and two photon polymerization (2PP). While cell transfer-based bioprinting technique is laser-induced forward transfer (LIFT). LAB techniques enable precise positioning of cells in 3D structure construct either by photopolymerization or cell transfer techniques.

Photopolymerization-based three-dimensional bioprinting techniques

Photopolymerization-based 3D bioprinting technique uses biocompatible polymer bioink in liquid form in the presence of photoinitiators that get photopolymerized upon exposure to light source of various wavelengths. These bioprinting techniques include SLP, DLP, and 2PP.

Stereolithography

Stereolithography (SLA) is the first and the most commonly used light-based bioprinting technique that fabricates the 3D structure construct with high resolution and accuracy. SLA is a nozzle-free technique based on curing of photopolymer resin using ultraviolet laser beam. Initially, the resin is in a liquid state at room temperature, which is then converted into the 3D object from geometric data obtained from a CAD file. Hull (1984) developed the process of photocuring for the first time, which was later commercialized. Here, the laser beam scans the bioink for purpose of curing. Once the first layer is solidified, the building platform moves to bring a new layer of bioink plastered on top, and the photocuring process is repeated. The layers are added to each other and the entire object is finally developed. Through this technique, 3D objects having complex structure can also be printed. A schematic diagram of SLA-based 3D printing system is given in [Fig. 1.5](#). In SLA bioprinting, bioink containing cells and hydrogel are photocured using a laser with a specific wavelength. The major challenge of SLA-based bioprinting is that the cells are damaged due to ultraviolet laser beam and photocurable bioink ([Duan, Hockaday, Kang, & Butcher, 2013](#)). The two other approaches of photopolymerization are vector-wise or direct laser writing and mask irradiation or mask-based writing.

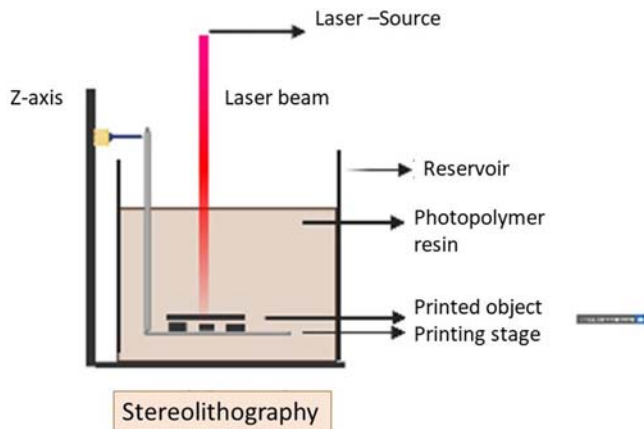


Figure 1.5 Schematic diagram of stereolithography-based three-dimensional printing system.

Digital light processing

Digital light processing (DLP) technique is similar to SLA just with a difference in light source. This technique requires 2D image from digital micromirror device (DMD) to polymerize an entire pattern using a single projected image. This technology uses a projector located at the bottom of the bioink pool. The DMD consists of micro-sized mirrors for fast and precise projection of UV/Visible light that facilitates rapid generation of bioprinted construct. The projector helps cover the entire surface of the layer leading to rapid bioprinting. The DLP technique allows fabrication of the construct in a layer-by-layer fashion resulting into higher resolution printing compared to SLA. In DLP, the projector resolution is directly linked to the print quality of the fabricated construct. This feature of DLP bioprinting technique is suitable for fabricating various 3D microstructures, such as hydrogel scaffolds in drug delivery systems, artificial tissues, and biomedical devices. A working diagram of DLP 3D printing technique based on DMD projector is given in Fig. 1.6. The DMD-projection bioprinting technique is capable of printing bioinks with high degree of precision (Huang, Qu, Liu, & Chen, 2014). Such LAB technique has recently attracted much attention due to its superior printing speed, high resolution, and high cell viability (Zheng et al., 2021). This technique is also suitable for multiple-tissue reconstruction or repair such as spinal cord, peripheral nerve and blood vessel injury (Bracaglia et al., 2017). Here, cell viability increases beyond 85%–95% due to short printing time and nozzle-free printing approach.

Two photon polymerization

2PP is a non-linear bioprinting process similar to SLA, but with a different photopolymerization process. In SLA, photopolymerization occurs at the resin surface while in 2PP it occurs into the resin. In 2PP, the two photons are absorbed simultaneously by the photoinitiator enabling them to act as one photon but with twice wavelength to effect fast polymerization. In this case, polymerization occurs in a

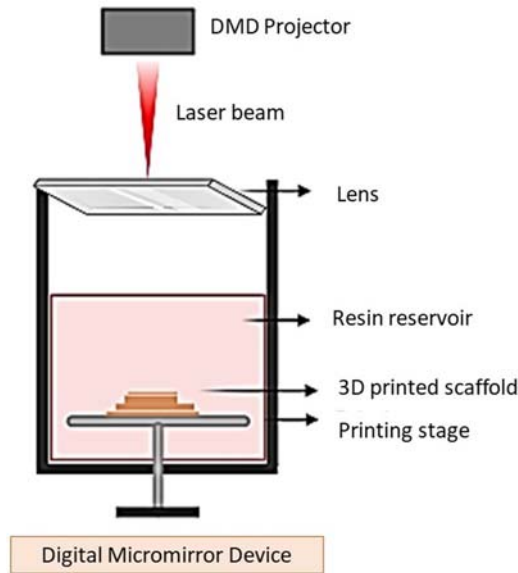


Figure 1.6 Schematic diagram of digital light processing three-dimensional printing technique based on digital micromirror device projector.

small region without disturbing nearby areas. This allows the laser beam to directly fabricate the desired 3D bioconstruct. A schematic diagram of 2PP-based lithography is given in Fig. 1.7. In addition to printing cells, the 2PP technique is also used to develop microfluidic devices and scaffolds for tissue engineering. This technique uses direct laser writing to fabricate 3D structure layer by layer by moving the focal point of the laser into the resin or bioink as the case may be. However, it takes a long build time to fabricate 3D structures with superior spatial submicron resolution (Faraji et al., 2021). At present, the high resolution of printed construct is linked with low throughput, which poses a major challenge with the currently available 2PP 3D bioprinters. Compared to other techniques, some scaffolds produced through this technique also lack biodegradability and biocompatibility.

Cell transfer-based, laser-induced forward transfer three-dimensional bioprinting technique

Laser-induced forward transfer (LIFT) bioprinting system involves the use of a pulsed laser beam, such as ultraviolet wavelength with nanosecond pulse, a gold or titanium-based ribbon structure serving as support for the bioink and the receiving substrate. The principle of LIFT is based on focusing of pulsed laser beam at the absorbing layer of ribbon structure during printing that results in evaporation and formation of high-pressure bubbles, propelling the cells containing bioink toward the printing receiving substrate (Barron, Ringeisen, Kim, Spargo, & Chrisey, 2004; Keriquel et al., 2017). The schematic diagram of laser-induced forward transfer bioprinting

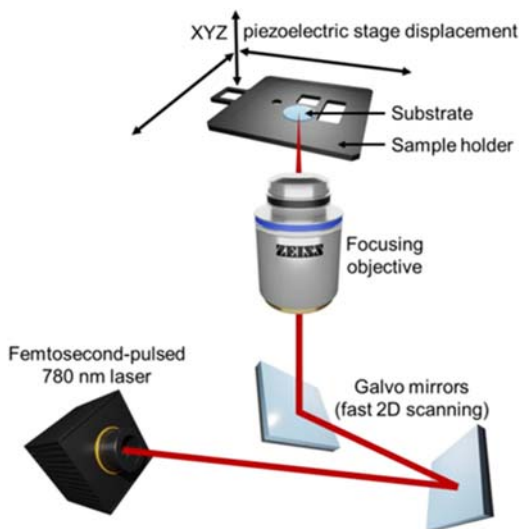


Figure 1.7 Schematic diagram of two-photon polymerization-based lithography (Bunea et al., 2021).

Source: Reproduced with permission from Bunea, A.L., Iniesta, N.C., Droumpali, A., Wetzel, A.E., Engay, E. & Taboryski, R. (2021) Micro 3D printing by two-photon polymerization: Configurations and parameters for the nanoscribe system, *Micro*, 1(2) 164–180, <https://doi.org/10.3390/micro1020013>.

technique is given in Fig. 1.8. This technique has been successfully applied to biological materials, such as polypeptides, DNAs, and cells (Ringeisen et al., 2004). The contact area after receiving the laser energy establishes high vibrational state and releases heat that causes the evaporation of bioink resulting into the formation of a droplet, which is then deposited on the receiving substrate (Gu, Fu, Lin, & He, 2020).

Droplet-based bioprinting

Droplet-based bioprinting techniques generate droplets of bioinks for fabricating biological constructs. These techniques include inkjet bioprinting, acoustic droplet ejection—based bioprinting, and microvalve-assisted bioprinting.

Inkjet bioprinting

Inkjet bioprinting technique uses droplets formed from bioink to fabricate the 3D construct. Today, this technique is popularly used to fabricate scaffolds and deposit cells. It is classified into two groups based on the droplet generation mechanism, namely continuous inkjet (CIJ) bioprinting and EHD jet or drop-on-demand (DOD) inkjet bioprinting (Derby, 2010). In CIJ bioprinting, the pressure is applied to force the bioink through a nozzle, which subsequently breaks into a stream of droplets to minimize its potential energy and surface tension (Leberfinger, Moncal, Ravnic, & Ozbolat, 2017).

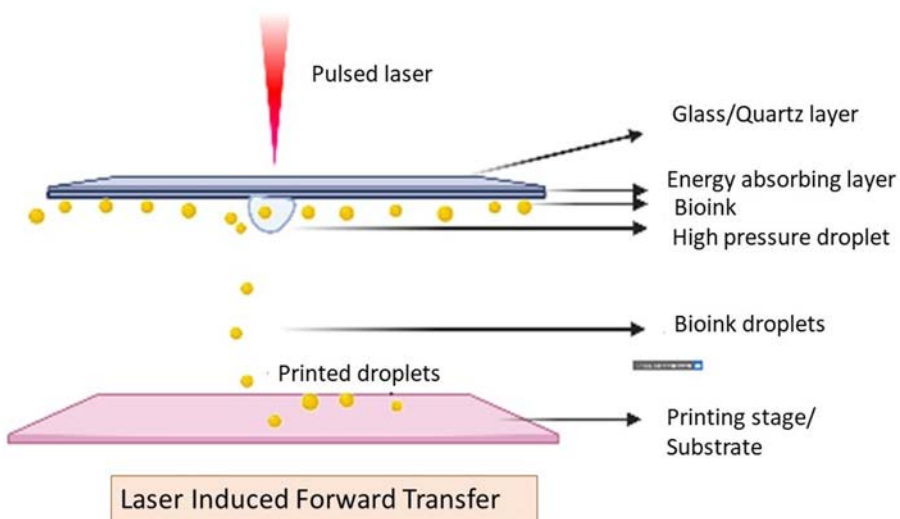


Figure 1.8 Schematic diagram of laser-induced forward transfer three-dimensional bioprinting technique (Serrano-Aroca, Vera-Donoso, and Moreno-Manzano, 2018).
 Source: Reproduced from Serrano-Aroca, Á., Vera-Donoso, C., Moreno-Manzano, V. (2018). Bioengineering approaches for bladder regeneration. *International Journal of Molecular Sciences*, 19(6), 1796.

Here, continuous drops are produced and remaining bioink is recycled, while in DOD inkjet bioprinting, the individual drops of 25–50 μm in diameter range are generated. DOD bioprinters are commonly used for tissue engineering applications. It is basically a noncontact technique wherein droplets of the bioink are propelled onto a printing stage by thermal, piezoelectric, or electrostatic mechanisms. Due to high cell viability, thermal deposition techniques are commonly used in biotechnology. EHD jet printing is mainly used to produce microfibers and scaffolds that support cell attachment. Inkjet bioprinting is flexible and inexpensive; however, it has problems such as nozzle clogging, slow printing speed, and low resolution. A working diagram of inkjet 3D bioprinting is given in Fig. 1.9.

An antioxidant colorimetric assay, where test substances could be evaluated through inkjet-printed enzyme inhibition assays has been reported (Lee, Samson, & Song, 2017). Inkjet bioprinting has been widely used to directly deposit various bioinks containing DNA or protein or nanomaterials. In particular, the inkjet bioprinting technique is appropriate for printing of those bioinks, where maintaining the native conformation of biomaterials during the printing process is necessary.

Acoustic droplet ejection–based bioprinting

Acoustic droplet ejection–based 3D bioprinting technique relies on the principle of acoustic field that helps eject droplets of bioink through a nozzle (Gudapati, Dey, & Ozbolat, 2016). The droplets are ejected through the waves created at the

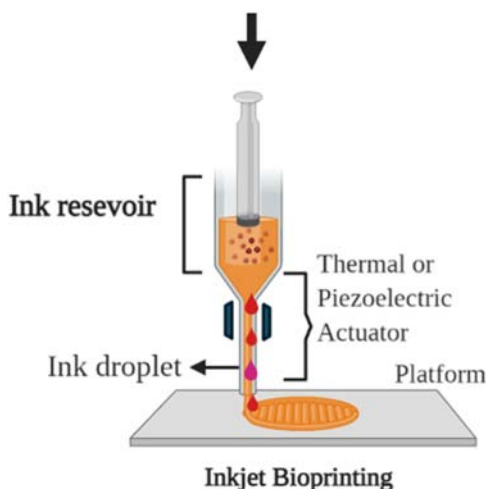


Figure 1.9 Schematic diagram of inkjet-based bioprinting.

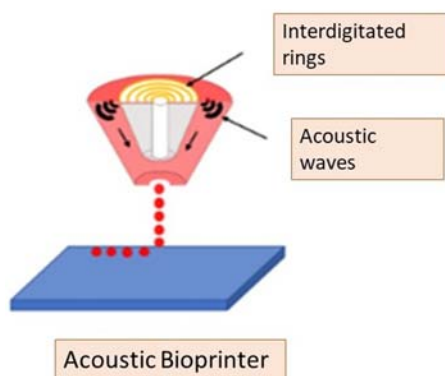


Figure 1.10 Schematic diagram showing droplet-based acoustic bioprinter.

Source: Reproduced from permission of Yu, J., Park, S. A., Kim, W. D., Ha, T., Xin, Y. Z., Lee, J., & Lee, D. (2020). Current advances in 3D bioprinting technology and its applications for tissue engineering. *Polymers*, 12(12), 2958. <https://doi.org/10.3390/polym12122958>.

interface between the air and the bioink. It is a quick, easy, and nozzle-free technique and does not exert mechanical stress on cells during the printing process. As bioink remains in an open pool rather than in a nozzle, it eliminates the exposure of cells to adverse effects of heat, high pressure, and high voltage. A schematic diagram showing droplet-based acoustic 3D bioprinting technique is given in Fig. 1.10. Being a nozzle-free technique, it is more suitable for fabrication of constructs having high-concentration of cells or even cell spheroids. Acoustic bioprinting offers higher cell viability compared to inkjet and EBB (Chen et al., 2021).

Microvalve-assisted bioprinting

Microvalve-assisted bioprinting technique is based on solenoid valve that converts electrical energy into mechanical energy. It consists of pressure source, ferro-magnetic plunger, and solenoid valve. The magnetic field generated across the solenoid due to electric pulse helps in the movement of magnet and coils and thus forces the plunger to move upward or inward. The upward motions open up the valve, which leads to the generation of droplets of bioink. A schematic diagram showing microvalve-assisted 3D bioprinting technique is given in Fig. 1.11. It is a reliable, inexpensive, and secure method of bioprinting that operates with interchangeable electromechanical or solenoid valves. Microvalve print head is able to dispense nanoliters to several microliters of bioink (Gudapati et al., 2016). As high shear stress on cells during droplet ejection is limited, therefore, this bioprinting technique provides high cell viability (Okubo, Qureshi, Dalgarno, Goh, & Derebail, 2019). Nowadays, microvalve bioprinting is used as a promising tool in tissue engineering (Dusserre et al., 2021).

Application of three-dimensional printing in various sectors of biotechnology

3D printing of biological materials compared to nonbiological materials involves additional complexities, such as the material choice, cell types, cell growth, cell

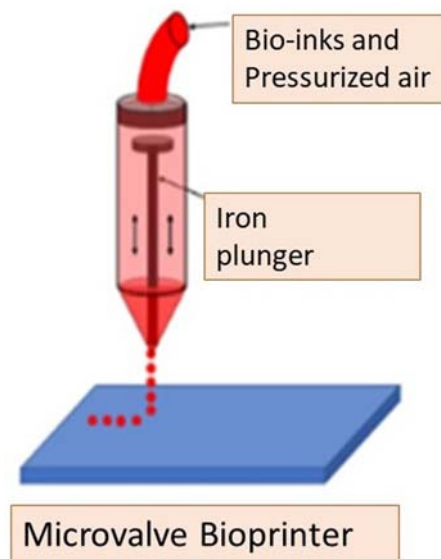


Figure 1.11 Schematic diagram showing droplet-based microvalve bioprinter (Yu et al., 2020).
Source: Reproduced from permission of Yu, J., Park, S. A., Kim, W. D., Ha, T., Xin, Y. Z., Lee, J., & Lee, D. (2020). Current advances in 3D bioprinting technology and its applications for tissue engineering. *Polymers*, 12(12), 2958. <https://doi.org/10.3390/polym12122958>.

viability, and problems related to the sensitivities of living cells and the construction of functional tissues (Murphy & Atala, 2014). Addressing these challenges requires knowledge of integrated technologies from various fields, such as engineering, biomaterial science, cell biology, physics, and medicine. Nowadays, 3D bioprinting techniques have applications in multilayered skin, bone, vascular grafts, tracheal splints, heart tissue, and cartilaginous structures. The emerging paradigm of 3D bioprinting in various fields, such as microbial cell printing, plant cell printing, and microfluidics is in blooming stage for the scientific community. The developments in high-throughput 3D bioprinting technology in fabricating tissue models for drug discovery and toxicology studies is transforming the biological and biomedical research. Some of the applications of 3D bioprinting are given below.

Tissue and organ fabrication

Tissue engineering has great potential in meeting the requirements of on-demand tissues and organs. The bioprinted biomimetic constructs with desired composition, better cell viability, and architecture that support the functionality of tissues and have the ability to overcome the organ shortage, such as vasculature, heart, liver, cartilage, bladder, and skin. The EBB method has been reported to fabricate tissue constructs, such as mandible, calvarial bone, cartilage, and skeletal muscles (Kang et al., 2016). The vascularization of tissue construct, which was previously not possible with conventional tissue engineering techniques, is now a possibility to some extent. Although, at present, bioprinted in vitro large tissues face challenges in terms of resolution, nutrient diffusion, and waste removal. Therefore, there is need to devise suitable strategies to support vascularization of tissue constructs particularly focusing on design of scaffolds with specific porosity, delivery of growth factors, cytokines, and surface immobilization of peptide sequences that accelerate angiogenesis. Furthermore, bioprinted construct of prevascularized engineered tissue requires optimization of various components of bioink.

Chen et al. (2020) recently reported multiple-tissue reconstruction or repair, including spinal cord, peripheral nerve, and blood vessel injury. Graham et al. (2017) demonstrated the bioprinting of human embryonic kidney cells (HEK-293T9) and ovine mesenchymal stem cells (oMSCs). The bioinks prepared by harvesting and dispersing cells are used for droplet-based 3D bioprinting technique, where mammalian cells are incorporated inside the droplet. The printed cellular construct is thus formed submerged in oil and subsequently can be transferred to aqueous culture, where it can develop cartilage-like structure with high precision and resolution.

Three-dimensional-printed organoids

Organoids are miniature forms of adult organs formed from multipotent and pluripotent stem cells. Organoids can be fabricated by bioprinting of either undifferentiated pluripotent cells or differentiated stem cells (Li et al., 2021). Organoids are composed of cell aggregates, so to fabricate organoids, it requires bioink containing

either homocellular cells or heterocellular cell types. 3D bioprinted hepatic organoids show liver functions such as albumin secretion, drug metabolism, and glycogen storage both in vitro and in vivo (Yang et al., 2021). The biofabrication of 3D tissue construct by precise positioning of cells in a controlled microarchitecture enhances tissue functionality. Such advancements in bioprinting techniques addresses the challenges of lack of precise architecture and large-scale tissue sizes for organoid fabrication.

Over the past years, 3D bioprinted organoids have gained great interest in the development of disease models and drug screening and toxicology testing and currently being explored more in personalized medicine and drug discovery. Reid et al. (2019) reported the use of customized low-cost extrusion-based bioprinter to develop 3D in vitro mammary organoids and chimeric organoids in collagen. These organoids helped investigate the molecular mechanism behind microenvironmental control of breast cancer in humans. Gu et al. (2020) performed the extrusion printing of human iPSCs with bioink containing alginate, carboxymethyl-chitosan, and agarose. The cells show differentiation in situ, self-assembled into three germ layers that demonstrate the maintenance of pluripotency after bioprinting. Nowadays, 3D bioprinting of human organs is being attempted, which include the heart, kidneys, lungs, gastrointestinal tract (intestine–gut–stomach), liver, placenta, adipose, retina, bones, and brain. Today, multiorgan models are also being explored using multipotent and pluripotent cells.

Another major development in organoid bioprinting is the development of vascular or perfusable structure using sacrificial inks. Sacrificial inks are soluble in water and can be easily removed from construct under specific temperature. They are easily extruded in the form of solid tubular structures by extrusion bioprinting (Gungor-Ozkerim, Inci, Zhang, Khademhosseini, & Dokmeci, 2018). These vascularized organoids can be used for organ transplantation, eliminating the need for an organ donor.

Organoid-on-chip

3D bioprinting along with the cellular self-organization approach can facilitate the functionalization of organ tissues. The advancements in microfluidic technology allows the development of organoids with structural and physiological feature in a controlled manner. For real-time monitoring, actuators and sensors can be integrated with micro-nanofluidic devices. It is now possible to recreate the structural and functional features of human organs using micro-nanofluidic devices. Karzbrun, Kshirsagar, Cohen, Hanna, and Reiner (2018) reported the folding mechanism of brain with the help of microfabrication of organoid-on-chip and further observed convolutions after organoid reached certain density. These organoids can also help in the study of fetal brain development. Kasendra et al. (2018) developed an intestinal organoid model, where the intestinal villi-like structure was formed in situ. Wang et al. (2018) developed liver organoid from embryoid bodies derived from hiPSCs, which undergo hepatic differentiation and maturation in situ in a confined 3D space. Therefore, today, the scientific community is engaged with the ultimate

goal of developing microfluidic multiorganoid systems so as to achieve *in vivo* like settings that capture the structure and physiology of different organ systems and recapitulate interorgan interactions and crosstalk.

Organ-on-chip

The organ-on-chip (OoC) models provide opportunity to understand the cause of disease and also serve as a model for drug screening. OoC provides 3D extracellular environment that closely mimics native extracellular matrix (ECM). Therefore, OoC is able to demonstrate the drug responses in a better manner, while providing the relevant information about the prevailing disease conditions. Over the past few years, several models such as liver on chip (Lee & Cho, 2016; Robbins, Gorgen, Min, Shepherd, & Presnell, 2013), heart on chip (Fleischer, Shapira, Feiner, & Dvir, 2017; Lind et al., 2017), lung on chip (Huh et al., 2010) and kidney on chip (Musah et al., 2017) have been developed. In recent past, effort has also been made to develop body-on-a-chip microfluidic perfusion system to integrate multiple functional organs, including heart and liver (Skardal, Shupe, & Atala, 2016). The tumor-like organoids such as tumoroids are derived from primary tumors to investigate the molecular mechanisms behind the cause of cancer and its growth, and further devise a strategy for effective targeted therapies (Tatullo et al., 2020). It is expected that this technology shall significantly improve the drug efficiency and lower the cost of the drug discovery. However, attaining high-throughput and optimizing suitable culture media are major challenges to the application of the body-on-a-chip. The development of organ-on-a-chip through integration of bioprinting technologies and microfluidics can serve as an ideal tool for studying the mechanistic insight of biological systems and drug screening to facilitate the observation of metabolism or secretion of organ models. These model systems offer sophisticated control of dynamic microenvironment, which overcomes the limitation of high variability of shape and size of organoids. The OoC offers the advantage of culturing living cells in a continuously perfused environmental chambers to model the physiological functions of tissues and organs by controlling the medium fluids containing cells and biomolecules (Mark et al., 2010). Here, the purpose is not to build a whole living organ system but just to synthesize minimal functional units that can recapitulate tissue and organ level functionality (Bhatia & Ingber, 2014; Richard, Richard, Neild, Cadarso, & Cadarso, 2020). The microfluidic print heads provide a micro-nanoscale reaction chamber to capture increased structural complexity and microarchitectural control as required to print 3D vascularized hollow structure to serve as blood vessel through cell-laden multilayer filaments. Lee et al. (2019) used multiinlet microfluidic print head with coaxial flow to fabricate fibrin-based model of glioblastomamultiforme, a deadly brain cancer, for drug screening. Snyder, Son, Hamid, Wu, and Sun (2016) demonstrated the application of microfluidic printing nozzle with laminar flow to print several materials.

It is evident that application of 3D printing in microfluidic devices is a comparatively new approach that could aid in understanding microbial mechanisms, exploring microbial consortia and microbe-microbe, and plant-microbe interactions. Microfluidic devices also allow researchers to explore both contact-based and

contactless interactions. It has the potential to become a gold standard analytical tool in future, which can reach its full potential when used in conjunction with regular lab procedures. The utilization of 3D printing for fabrication of microfluidic device will be extremely beneficial for biological and medicinal applications. In the coming years, 3D printing is anticipated to become a routine tool for organ-on-a-chip engineering.

Recent advances in bioprinting and 3D cell culture have revealed novel methods for modeling microbial infections, host-pathogen interactions, microbiota niches, biofilm formation, and determining antibiotic resistance. Spheroid/organoid cultures, explant/organotypic cultures, polymeric scaffolds, natural and synthetic hydrogel scaffolds, and microfluidics are the most commonly documented approaches for modeling host tissues and understanding the mechanism of disease initiation.

Food printing

3D-printed food has emerged as a novel food based on designing and customizing food materials with complex geometry, detailed patterning, and proper nutritional value (Godoi et al., 2016). 3D food printing offers personalized nutrition, automated cooking, and reduction in food wastage. This revolutionary technology has also shortened the supply chain and broadened source of available food materials, including meat (Attarin & Attaran, 2020). The global food industry has started banking on it to fulfill the unmet requirement of personalized nutrition. However, there is still very few mass production, despite its potential advantages in terms of flexibility, precision, low waste generation, and capability to design food of choice. At present, 3D food printing at industrial level is limited due to high cost, long time, and lack of demand for production at a large scale.

The potential applications of 3D food printing ranges from domestic kitchens, restaurants, and shopping malls to spaceships. With time, the associated ingredients involved in 3D food printing is expanding from naturally printable food ingredients, such as sugar, chocolate, hummus, and cheese to more healthier and sustainable food ingredients, including plant-based protein. In a globalization era, the food supply chains for shipping of preprocessed food from a factory to different parts of the world are increasingly becoming complex and less transparent. Although 3D food printing at present is in its nascent stage, it has the potential to address many pressing challenges of traditional food production practices as well as food security. The conventional food value chains involve mass standardization of food products that often results in over exploitation of resources, high carbon emissions, and food waste. The 3D-printed food has the potential to make the existing food value chains more sustainable and consumer-friendly by facilitating on-demand food availability, reducing food wastage, and enabling automated food personalization. It can also help customize the ingredients of food products to suit the individuals health and taste preference requirements. There is still a long road for 3D food industry to penetrate the major segment of society. Thus far, its use is still limited to the academic arena, catering, and confectionery sectors. Most of

the existing 3D food printing systems are not designed for large volume applications and lack functional value propositions and well-defined customer segments as well as IPR and profit mechanisms.

Biomedical implants and anatomical models

Sachs, Haggerty, Michael, & Williams (1993) patented a 3D printing technique that served as the basic idea behind the first 3D printing method used in the development of pharmaceutical dosage forms. 3D printing has potential in fabrication and production of complex and interconnected porous structures as per the requirements of patient-specific customized implants at relatively low cost. Furthermore, it allows fabrication of structures that have sites-specific mechanical and physical properties as well as spatial and temporal control of bioactive components. Prosthetic implants can be made of any desired geometry through translation of X-ray, MRI, or CT scans into digital.stl files (Banks, 2013; Gross, Erkal, Lockwood, Chen, & Spence, 2014; Klein, Lu, & Wang, 2013).

Today, 3D printing is transforming the biomedical and medical devices industry and has made it possible to fabricate both standard and complex customized prosthetic limbs and surgical implants within a day (Banks, 2013; Hod Lipson, 2013). It is a fact that BIOMED Research Institute, Belgium successfully implanted the first 3D-printed titanium mandibular prosthesis. This implant was developed by successively melting the thin layers of titanium powders using a laser beam. In 2013, the first 3D-printed polyether ketone ketone (PEKK) skull implant was developed by Oxford Performance Materials. Nowadays, 3D printing offers to fabricate light-weight, low-cost, and easily replaceable prosthetic implants for children (Burn, Ta, & Gogola, 2016).

The inability to communicate with the brain in terms of sensibility has been the major limitation of prostheses. The bioprinted cellular prostheses have potential to overcome this limitation. Nylon-based uretic stents and laparoscopic trocars were printed and successfully developed in a female cadaver and in vivo porcine model (Del Junco et al., 2015). Congenital or traumatic deformities of ears are commonly replaced by silicone prosthesis or patients' cartilage; however, it is expensive and usually involves several hospital visits. Moreover, it is difficult to obtain the shape that perfectly fits to the defect site without additional fillers or resection of healthy tissues. The 3D-printed ears were reported in 2014 (Lee et al., 2014). Today most of the hearing aids that fit into the ear are customized by 3D printing. The potential applications of 3D printing may include personalized, presurgical, and preoperative planning that will lead to reduce a multistep procedure and might help determine the best therapeutic option. Over the past few years, several studies have demonstrated that patient-specific presurgical planning may reduce the time spent in the operation room that in turn may result in fewer complications (O'Brien, Wayne, Barsness, McGaghie, & Barsuk, 2016; Perica & Sun, 2017). Moreover, physical 3D model of the desired patient anatomy could enable a surgeon to accurately plan the surgical procedure based on patient-specific anatomy. Furthermore, 3D printing also provides the possibility to choose the size of the prostheses components with very high accuracy (Aimar, Palermo, & Innocenti, 2019).

Drug delivery and drug development

Over the past decades, advancements in pharmaceutical sciences and bioengineering have led to the personalization of therapies. 3D printing is an emerging technique that has the potential to create complex and customized drugs and manufacture pharmaceutical dosage according to patient need. The fused deposition modeling 3D printing technique is versatile and an economical approach to produce complex designs with precision of quantity and the ability to incorporate various infill densities. Hotmelt extrusion technique, an FDM 3D printing approach that require filaments as ink roll or feedstock material is used in the pharmaceutical manufacturing (Dumpa et al., 2021). The 3D printing of drug involves powdered drug layer, so as to dissolve faster than average pills (Konta, García-Piña, & Serrano, 2017). These 3D-printed drugs allow personalization for patient-specific needs (Lee Ventola, 2014). 3D printing can also be used to produce polypills by combining multiple dosages into a single formulation or can be rapidly manipulated by physically modifying the tablet dimensions or infill density. Changing the physical dimensions of printed drug helps in studying a wide dose range. This may further provide an easier, more efficient, and precise means of dose evaluation and data collection. However, altering printed drug geometry may affect drug release. This technique may be used to rapidly produce formulations with dose flexibility, on demand, and at low cost that could expedite the early phase drug development, such as preclinical studies and first-in-human trials (Xu et al., 2021).

The emerging potential of 3D printing in producing personalized drug of precise measurement, customizable to patients need, has shown the possibility of developing personalized medicines of novel dosage forms. At present, recent advancements in 3D printing techniques, particularly used in the pharmaceutical industry, focuses on fabrication of different dosage forms.

Bioprinting in plant science

Green bioprinting is a new approach that helps explore plant–microbe interaction at cell, tissue, and whole plant. Mehrotra, Kumar, Srivastava, Mishra, & Mishra (2020) provided the concept of 3D bioprinting in plant science and discussed for the first time the bioprinting of plant cell itself and further proposed and emphasized to recreate precise and safe intricate microarchitectures of plant organs through bioprinting techniques that can mimic natural biological function of plants. 3D bioprinting can develop plant cell-based and plant-inspired 3D constructs. These constructs may help answer many unsolved queries in plant science in terms of metabolites production, therapeutic protein production, disease resistance mechanism, and plant–microbe interaction. Beckwith, Borenstein, & Velásquez-García (2021) demonstrated a the first proof-of-concept of bioprinting of *Zinnia elegans* cells using gel-mediated plant cell culture. Lode et al. (2015) showed extrusion-based 3D plotting of a basil cell-laden hydrogel blend consisting of alginate, agarose, and methylcellulose (alg/aga/mc). Thus, green bioprinting allows the processing of live cells from the plant kingdom, including immobilization of plant cells

that enables the development of new bioprocesses for secondary metabolites production. Plant bioprinting can also potentially revolutionize many applications, such as production of plant-based materials and designer plant-based food (Choudhury, Anand, & Naing, 2018). Furthermore, there is need to develop technologies that use plant cells for large-scale manufacturing of plant organoids. First, 3D bioprinting-based medicinal plant cell cultivation approaches can transform herbal medicine sector. Second, biomass-based industries including bioenergy can be benefitted with this pioneer technology because cultivation of biomass is dependent on many unpredictable factors, such as flood and draught, etc.

Microbial cell printing

Microbial cell printing opens up a window of opportunity to explore the full potential of this technology in the development of highly cost-effective strategies for remediation and monitoring of natural samples. This emerging topic has made a paradigm shift in the fabrication of 3D objects. Particularly, in environmental biotechnology, bacteria-associated bioprinting finds applications in fabricating suitable biomaterial for microbial microenvironments to explore the microscale communications and quorum sensing with the help of biofilm formation (Connell, Ritschdorff, Whiteley, & Shear, 2013). Microbes are the key drivers of entire ecosystems and biogeochemical processes, but little is known about the 3D organization of these dynamic organisms and their overall function. 3D printing has enabled the materials to act as live materials by embedding them with living cells that have the ability of energy production, physical movement, perform sensing, and also in biological synthesis (González, Mukhitov, & Voigt, 2020). Dubbin et al. (2021) developed a technique for patterning microbes in 3D geometries using projection SLA to bioprint microbes within hydrogel architectures. 3D bioprinting of bioink containing living materials and other biomaterials have potential applications in removal of toxic compounds from the environment (Kyle, 2018). Schaffner, Rühls, Coulter, Kilcher, and Studart (2017) took two different organisms, *Acetobacter xylinum* (*A. xylinum*) and *Pseudomonas putida* (*P. putida*), to develop living materials for both bioremediation and biomedical applications, where *P. putida* acts as phenol degrader and is able to form an interface between air and water by secreting amyloid fibers. 3D-printed human cell-based scaffold models are suitable for studying quorum sensing and bacterial biofilm development. The design, formulation, and optimization of diverse bioinks to develop bioprinted construct having high cell viability and cellular distribution are expected to receive immense attention in the near future.

A detailed account of 3D printing applications in microbial systems is discussed in Chapter 5.

Bioprinting of nanomaterials

3D printing of nano biomaterials to accurately fabricate any desired 3D tissue model is gaining momentum due to its diverse applications in biological and biomedical fields. 3D printing is being explored for fabrication of nanomedicine,

which has a promising potential in fulfilling the need for patient-centric personalized treatment. The availability of novel natural biomaterials and precisely engineered polymeric materials can be fabricated into exclusive 3D-printed nanomaterials as nanomedicine. Nanomedicine is also showing great impact in the design and development of precision medicine. The conventional medicine system of one-size-fits-all criterion failed to deliver the requirements of individualized treatment, while personalized or precision medicine considers the differences in various traits, including pharmacokinetics and genetics of different patients, which have shown improved results over conventional treatment methods. Over the past years, rapid progress has been made in the development and design of tailor-made nanomedicine using 3D printing technology (Jain, Shukla, Yadav, Ujjwal, & Flora, 2021). The fabrication in micro/nanoscales may change the performance of biomaterials and devices because it can retain more anisotropy of materials compared with the traditional rapid prototyping techniques (Liu et al., 2013). Nanomaterials can help alter mechanical, optical, electrical, thermal, actuation, and biological properties of 3D-printed devices as per specific requirements. The integration and manipulation of nanomaterials in 3D printing process can impart tunable and functional properties to the developed construct. This multiscale patterning enables customization of the form and function of 3D-printed architectures for its wider applications in biotechnology.

Conclusion

3D printing in biotechnology is an emerging area with huge potential for researchers and industry. At present, it is in the nascent stage. Speed of research progress in healthcare, cosmetics, and food is faster compared to other application domains due to obvious reasons. 3D printing applications in applied microbiology and bioremediation is just beginning to be recognized. 3D printing applications in plant science has begun very recently. 3D bioprinting has achieved significant advances in tissue engineering and biomedical applications. It has the potential to develop artificial organs for human transplantation. Hydrogels and tissue implants have a growing market. Research and development on micro-nanofluidic devices for point-of-care diagnosis is on the rise.

Despite these advantages, there are still some pertinent concerns that need to be addressed.

The developments in 3D bioprinting technology have provided hope for better disease diagnosis and treatment including personalized medicine for variety of diseases; however, printing resolution, speed of printing, cost of bioprinters, and availability of novel bioinks are still major challenges.

The components used to make bioink should be biologically active and have suitable interactions with the cellular components, as well as gain controlled mechanical qualities after printing (Murphy, De Coppi, & Atala, 2020). Another option is to create hybrid structures that use both synthetic and natural elements to give structural integrity and a cell-friendly environment for cellular growth. Synthetic peptides, such as Arg-Gly-Asp (RGD), found in the ECM environment,

can be used to control the mechanical characteristics and degradation time of material by crosslinking. Fortunately, hydroxyapatite with methacrylation via glycidyl-hydroxyl reaction and PEG-RGDS-containing bioink results in adequate stiffness and cell survival in case of retinal bioprinting (Wang et al., 2018).

For modeling viral and bacterial infections, we anticipate the integration of modular infectious units with various organs-on-chips, where infectious units can be added or deleted for a more realistic assessment of diseases and its effect on the concerned organs. Modeling virus infections has been done using 3D organotypic epithelial raft cultures, gut-on-a-chip, liver-on-a-chip, and dynamic cell culture vessels. Despite recent progress, more work is needed to make in vitro infection models a regular strategy in preclinical studies and tailored treatments in clinical settings. The development of 3D structures that can respond to external stimuli, such as pressure, heat, electric current, and ultraviolet light, etc. is of great importance due to various applications in bioengineering.

It is expected that the scientific community may be able to offer insights into future efforts to make these technologies more translational to help pharmacological and pathophysiological research more broadly. The emergence of multiple startup firms and rapid growth in the field of tissue and microbial engineering will accelerate the advent of such systems in real-world applications, for example, developing efficient tissue models for infectious disease control and elimination.

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Formulation of bioinks for three-dimensional printing in biotechnology

2

Chapter outline

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Introduction

Three-dimensional (3D) printing has gained a lot of attention due to its various advantages over conventional techniques. Bioprinting has made it possible to engineer tissue scaffolds with required structural properties such as strain, Young's modulus, and elasticity; permeability, porosity, and vasculature. Recently, much research has been focused for the development of bioink. Since handling of biological samples are sensitive to various environmental factors, such as pH, temperature and pressure, the bioink should be able to withstand not only these factors but should also be able to promote the growth of cells—bacterial, animal, or plant and maintain the structural integrity of the final printed product. Bioprinting of tissues or complete organs involves a delicate relation between the interaction of biomaterials with the live cells, motor movement, the type of printer used, and posttreatment applied (Gopinathan & Noh, 2018; Murphy & Atala, 2014).

Earlier, the inks of 3D printers were not meant for biological samples. The conventional inks were plastics, metals, alloys and thermoplastic polymers. These inks can withstand high temperatures and strong organic solvents. For the printing of

biological samples, natural polymers or biomaterials are preferred over other materials. Naturally derived polymers such as collagen, alginate, chitosan and fibrin have gained much attention mainly due to its similarity to provide the natural extracellular matrix (ECM) and biocompatibility (Lee, Park, & Koh, 2018). Some of the synthetic polymers, such as polyvinyl alcohol (PVA) and polyethylene glycol (PEG), have also been explored as bioink. The major advantage is the tunability of synthetic biomaterials. The properties of these biomaterials can be tailored according to the required application. However, the issues related with synthetic polymer are toxic degradation and poor biocompatibility, thus invoking immune response (Hospodiuk, Dey, Sosnoski, & Ozbolat, 2017). The pros and cons of each polymer are discussed in the following sections.

Bioinks can be broadly categorized in two approaches. One is the printing of scaffold-impregnated cells and second is scaffold-free cell approach. In the first approach, a biomaterial polymer is mixed with live cells and is printed layer by layer to form 3D structures (Kaushik, Kim, & Walma, 2016). The biomaterial erodes, surface erosion or bulk erosion, depending upon the type of biomaterial used. The cells derive nutrition from the surrounding tissues and differentiate to form tissue structures. The biomaterial provides the initial strength and integrity required to scaffold-laden cells. The second approach is the direct printing of cells in a specific structure and arrangement that can mimic the tissue. The requirements of bioink changes upon the application to be used and with the design of complex anatomical structures, the desirable properties of materials become more complex and specific. The materials must crosslink in a defined manner to hold the structure of deposited layer while maintaining the functional integrity of the printed scaffold. Fig. 2.1 highlights the requirements of a bioink for printing of tissues and organs. The inks used for 3D printing in food has been discussed in

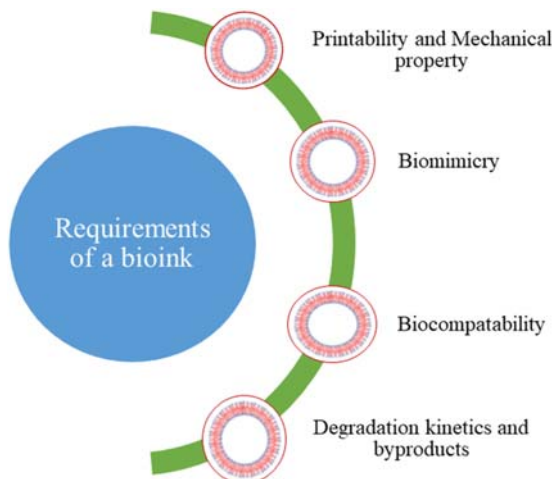


Figure 2.1 The requirements while designing of bioink.

Chapter 6 while for the materials used for printing of bacterial cells have been discussed in Chapter 7. The current chapter deals with the materials used for bio-printing of tissues and organs.

Materials for printing of biological samples

The different materials used as inks will be discussed in detail highlighting the requirements, advantages, disadvantages, and modifications done as per the current research.

Scaffold-based bioprinting

Metals and alloys

Metals and alloys are generally not termed as “bioink” as they cannot integrate biological cells. However, the inks derived from metals and plastics are used for personalized implant design, especially for bones and in dentistry. The basic process of 3D printing is same in both the cases—acquiring information about the physical model through CT scan, X-ray, or MRI; design approach and creation of STL file; selection of material—metals, ceramics, plastic, 3D printing; and finally postprocessing—heat treatment, jet washing, infiltration, grinding, or in combination. 3D printing has paved the way for development of personalized implants that fit better than molded implants. Popovich et al. fabricated a custom-made hip implant using selective laser melting printer. The metal powder was fused with the next layer using laser irradiation. Titanium alloys have high biocompatibility and better mechanical properties with easy osteointegration to form complex geometrical shapes (Popovich, Sufiiarov, & Polozov, 2016). Customized implants are beneficial because of the uniqueness of each defect, anatomical differences, presence of vital healthy tissues near to the affected areas, and chances of infection. A recent update of materials used for implants have been discussed in Chapter 3. The bioink comprising biomaterials encapsulating the live cells have been discussed in detail in the current chapter.

Polymers and composites

A wide range of polymers are used as bioinks depending upon the end application and location in the body—bone, nerve, skin, and cardiac cells. Natural and synthetic polymers have their pros and cons. However, not one single polymer can be used to print cells of different types. Research has also been focused upon blends of polymer and growing of co-culture systems. Generally, the bioinks used are the already reported polymers and rarely new biomaterials are reported. One of the major reasons is that majority of the work is being done in biology-based labs and the

discovery of material with good biocompatibility takes time (Valot, Martinez, Mehdi, & Subra, 2019). In this section, we discuss about the recent advances in polymers that are used as bioinks.

Hydrogel

Hydrogel can be defined as a hydrophilic polymer network that can absorb water many times than its own weight. The polymer solution has fluid-like property and is called as sols before they are crosslinked. This solution is required to be cross-linked to a solid state either by physical or covalent interaction. Hydrogels are highly biocompatible and biodegradable in nature, making them an excellent choice for scaffold fabrication. However, the major disadvantage with hydrogel is its weak mechanical strength, cell compatibility and mimicking the ECM environment of the cell. The major focus of hydrogel bioink is controlling the fluid properties of both noncrosslinked solution and after crosslinking the structure in which it is printed (Chimene, Kaunas, & Gaharwar, 2020). Due to advances in chemical methods of hydrogel fabrication, such as click chemistry, gelation mechanisms, and mixing with nanoparticles, it is now possible to control the physicochemical properties of hydrogel (Zhang & Khademhosseini, 2017).

A number of factors influence the printability of the hydrogel, such as the polymer compatibility, viscosity of the ink, gelation mechanism and time, fabrication time, shear stress, nozzle gauge, and network properties (Malda, Visser, & Melchels, 2013). Several naturally derived polymers have been explored, such as chitosan, alginate, gelatin, cellulose, and hyaluronic acid that are not only biocompatible but also shows good mechanical strength. From the synthetic counterpart, PEG has been widely used as a hydrogel bioink. Apart from the biocompatibility and mechanical properties as mentioned above, other factors that need to be taken into consideration are biodegradability, mass transport, target cell environment and effect of crosslinking reactions to the cell viability (Seliktar, 2012). Biodegradability is an important aspect when fabricating a scaffold. The scaffold should disintegrate without the formation of any toxic bioproducts and the encapsulated cells should proliferate to develop its own ECM. The bulk dissolution of the hydrogel creates pores that gives space for cell migration, proliferation, and infiltration of blood vessels. The spatiotemporal control at the cell material interface is challenging, as the natural ECM contains a variety of proteins, such as collagen, fibronectin, laminin, elastin, growth factors, inhibitors, enzymes, and polysaccharides (Kharkar, Kiick, & Kloxin, 2013).

Fabricating a 3D-printed hydrogel is greatly dependent upon the extent of crosslinking, the fabrication method, and the intermediate and degraded by-products. The free radicals generated, the initiators, or the monomers can be damaging to the cells. Apart from the chemicals involved, the UV light or blue light for photo-crosslinking can also be toxic to the cells (Berg, Hiller, & Kissner, 2018). Tissue formation greatly depends upon the mechanical strength

of the hydrogel. If the crosslinking network is high, high pressure is applied through the nozzle that may create shear stress for the cells and low viscosity hampers the structural integrity of the printed cells. The hydrogel scaffold should be strong enough to withstand the load of the cells until it creates its own ECM. The strength of the hydrogel can determine the differentiation of the encapsulated cells. Polymer hydrogels that are explored for 3D printing applications are discussed as follows.

Alginate-based hydrogel

Alginates are linear anionic polysaccharides that are derived from bacteria and algae. They have been widely used in tissue engineering as they can mimic the ECM of human tissues. They consist of linear (1–4)-linked β -d-mannuronic acid (M blocks) and its C5-epimer α -l-guluronic acid (G blocks) residues with a 4C1 ring conformation. They can form hydrogel by forming an ionic bridge between the adjacent polymer chains by interaction between the divalent cations and the G block monomers. Alginates are highly biocompatible in nature. However, the major limitation in its use is the low degradability, as mammals lack alginate-degrading enzymes (Reakasame & Boccaccini, 2018). Only low MW (less than 50 kDa) can excrete out through kidney while the high molecular weight is retained in the body (Unagolla & Jayasuriya, 2020). Alginates have been engineered with functional groups to tackle this problem.

Hong et al. fabricated a stretchable hydrogel with high mechanical strength using sodium alginate and PEG to form a hydrogel network. The hypothesis followed by the authors was the crosslinking of alginate with Ca^{2+} provides mechanical property while PEG crosslinking provides the elasticity under large deformed conditions. The fabricated hydrogel was found to be tougher than the natural cartilage. The cells encapsulated within the hydrogel showed a viability of upto 7days. Nanoclay also provided support to the hydrogel without any base material (Hong, Sycks, & Chan, 2015). Similarly, Leppiniemi et al. fabricated a nanocellulose–alginate hydrogel biofunctionalized with avidin protein to the cellulose nanofibrils using covalent coupling reaction. The crosslinking was done by calcium ions by ionic interactions. The mechanical property had good tissue compatibility (Leppiniemi, Lahtinen, & Paaanen, 2017).

Sodium alginate lacks cell-adhesive ligands and is therefore blended with other biopolymers, such as poly(γ -glutamic acid) or gelatin to improve the cell proliferation activity. Modifications is also required as alginate-based hydrogel also has low physiological stability and shape fidelity. Nanocellulose has also been explored due to its excellent mechanical properties, tailorable chemistry and biocompatibility. It also exhibits shear thinning property that is helpful for extrusion of bioink (Di Giuseppe, Law, & Webb, 2018; Zhu, Chen, & Feng, 2018). Wei et al. fabricated TEMPO-oxidized bacterial cellulose/alginate hydrogel with enhanced stability with nanoclay incorporation. The hydrogel maintained high shape fidelity. Nanoclay effectively improved the stability of the hydrogel and also the prolonged delivery

of proteins from the hydrogel (Wei, Wang, & Li, 2020). Sodium alginate also supports hyaline-like cartilage better than GelMA and BioINKTM as observed by Daly, Critchley, Rencsok, and Kelly (2016).

Collagen-based hydrogel

Collagen is an important component of ECM and one of the most abundant protein. Collagen exists in 29 distinct forms; however, out of I, II, III, V, and XI, collagen type I is the most studied since only this type can form fibrous hydrogel through self-assembly. It remains liquid at room temperature while forms gel as temperature rises to 37°C, however, the gelation time is low—it takes half an hour to form gel—making it difficult to retain the structure after bioprinting. Also, as the gelation time is low, the encapsulated cells tend to move downward due to gravity, thus affecting the homogeneity of the hydrogel. Thus, a support material is required to improve gelation rate as well as mechanical property. A number of hydrogel systems have been developed to incorporate collagen as a 3D printing bioink (Lin, Zhang, & Macedo, 2019; Sarriannidis, Rey, & Dobre, 2021).

Yang et al. fabricated collagen-alginate hydrogel to construct a 3D-printed tissue cartilage. It was observed that the hydrogel facilitated cell adhesion and increased proliferation of cells and cartilage-specific gene expression, such as Sox9, Col2a1, and Acan. The hydrogel also suppressed the dedifferentiation of the chondrocytes and maintained the phenotype of the tissue cartilage. Collagen also contains high density of RGD sequence, which enhances cell adhesion and its differentiation.

Chen et al. developed a gellan gum/collagen hydrogel that was used to encapsulate bone marrow-derived mesenchymal stem cells. The vinyl group of gellan gum and collagen was modified by a double crosslinking method that enabled solubility in water at room temperature. The 3D-printed structure had the required mechanical property and enabled vascular differentiation. The hydrogel also promoted the differentiation of bone marrow-derived mesenchymal stem cells to endothelial cells (Chen, Zhang, & Ding, 2018).

Polymers that mimic ECM characteristics have the limitation of excessive swelling, lack of self-healing and shear thinning property, and toxic cross-linkers, which limits its application in injectable hydrogels. Pupkaite's team fabricated collagen-based injectable hydrogel with controlled swelling behavior using thiol-Michael addition click reaction. Thiol groups were introduced in the collagen and cross-linked by 8-arm PEG-maleimide. The hydrogel showed only 6% swelling over a period of 1 month in aqueous buffer. The hydrogel was also biocompatible and showed proliferation of endothelial cells and mesenchymal stromal cells (Pupkaite, Rosenquist, Hilborn, & Samanta, 2019).

Collagen is also a promising candidate for bone tissue engineering research. Mineralization of collagen hydrogels with calcium phosphate is carried out using either freeze-dried scaffolds or injectable hydrogels, which are promising candidates for bone defect repair. During the bone formation process, phosphoproteins and alkaline phosphatase participate in the collagen mineralization, an important tool for fabricating bone repair scaffolds. Introduction of alkaline phosphatase and phosphoprotein is a major challenge. Chen and team prepared alkaline phosphatase

methacrylamide by amidation reaction, which was uniformly grafted upon collagen. The photo-crosslinkable gel achieved homogeneous enzymatic mineralization. They also incorporated a phosphoprotein (vinylphosphonic acid) and 3D-printed structure to have mechanical porosity and stiffness of the natural bone (Chen, Yang, & Zhao, 2019).

Chitosan-based hydrogels

Chitosan comes under the category of amino polysaccharide as it contains active amino groups attached, giving it cationic character. Chitosan is also a versatile biomaterial as new groups can be attached to the amino group using mild reaction conditions. Chitosan consists of copolymer of *N*-acetyl-D-glucosamine and D-glucosamine units with one amino (NH_2) group and two hydroxyl (OH) groups in each repeating glycosidic units. Chitosan is soluble at low pH as it becomes a polycationic species due to protonation of amino group. This cationic property can be reversed by sulfonation reaction to make it water soluble by introduction of anionic character. Depending upon the fabrication method, the molecular weight varies from 300 to over 1,000 kD (Ali & Ahmed, 2018).

Recently, self-healing hydrogels are being fabricated for their use as bioinks for printing of cells/tissues. Self-healing hydrogels should maintain the structural integrity during the printing process and also be stable during the post-printing process. Liu et al. developed an injectable hydrogel by functionalizing chitosan with phenol group and dibenzaldehyde-terminated telechelic poly(ethylene glycol). Phenol functionalization helps increase gelation time, visible light crosslinking capacity, self-healing ability, and long-range critical gel behavior. As shown in Fig. 2.2, the hydrogel was successfully 3D-printed into individual blocks and then crosslinked by photo-crosslinking (Liu, Wong, Chang, & Hsu, 2021).

The development of chitosan inks generally involves the use of organic solvents. These solvents may create toxicity or toxic by-products. Zhou et al. formulated a chitosan ink by dissolving in an alkali aqueous solution, which is at solution state at low temperature but undergoes gelation by self-assembly at higher temperature. As the chitosan ink is extruded in hot water, the chitosan ink gels into a stable structure. A direct ink method was developed to provide stable structure printing (Zhou, Ramezani, & Sun, 2020). Similarly, the fluid property improved after mixing with hydroxyapatite with chitosan hydrogel with osteoblast cell line MC3T3-E1 (Demirtaş, Irmak, & Gümücsderelioğlu, 2017).

Chitosan hydrogels are soft hydrogels with limited mechanical properties. Chitosan is required to be mixed with another biomaterial to improve its mechanical property. Wu et al. fabricated a chitosan hydrogel which is highly flexible and have an organized microfiber networks. The micro-organized structure can guide and align cell growth, which is an important factor in producing 3D structures (Wu, Maire, & Lerouge, 2017). The fabricated ink did not go upon shrinkage in volume and maintains the desired morphology. Chitosan has an excellent ability to form pH-induced hydrogels; however, due to high water content it has low stiffness or mechanical strength. Shrinkage in such hydrogels can be overcome by the use of filler materials, so that shape integrity can be maintained. To overcome this, silk

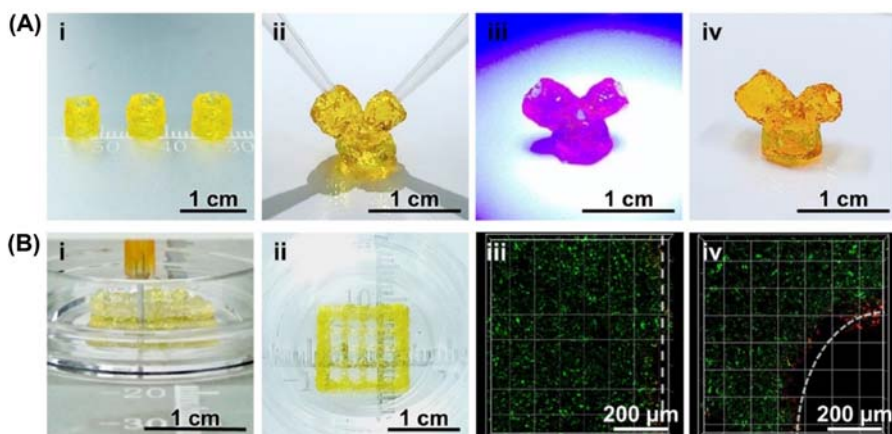


Figure 2.2 3D printing of phenol functionalized chitosan hydrogel (A) (i) 3D printing of individual components, (ii) photo-crosslinking of the hydrogel, (iii) irradiated with blue light, and (iv) final printed product. (B) (i) 3D printing of human mesenchymal stem cells encapsulated hydrogel, (ii) top view of the structure, (iii) confocal microscopic images showing live and dead cells, the borders are indicated by dashed line, and (iv) 3D confocal microscopic images showing the live/dead staining of hMSCs in the filament of the construct after 4 h.

Source: Reproduced with permission from Liu, Y., Wong, C.-W., Chang, S.-W., & Hsu, S. (2021). An injectable, self-healing phenol-functionalized chitosan hydrogel with fast gelling property and visible light-crosslinking capability for 3D printing. *Acta Biomaterialia* 122:211–219.

particles can be used, which not only has excellent biocompatible property but also can maintains the fracture toughness, creep resistance, and stiffness of the printed material (Zhang, Allardyce, & Rajkhowa, 2018).

Gelatin- and methacrylated gelatin–based hydrogels

Gelatin is water soluble and biodegradable in nature. It is a partially hydrolyzed form of collagen. Gelatin degrades rapidly because of its high solubility and undergoes enzymatic degradation. The gelling mechanism also depends upon the source—such as fish-derived gelatin or mammalian-derived gelatin. Gelatin-derived hydrogels have poor mechanical strength and find it difficult to maintain the shape integrity (Raucci, D’Amora, & Ronca, 2019). Since crosslinking takes time and under extreme conditions, encapsulation of cells have been restricted. Gelatin requires filler material to improve its strength. A lot of research has been focussed for incorporation of both natural and synthetic polymers to improve its strength.

In a study by Jiang et al., gelatin was mixed with dialdehyde cellulose nanocrystals to improve the mechanical strength of the hydrogel. Dialdehyde cellulose nanocrystals naturally crosslinks the hydrogel by a Schiff base reaction. The mechanical strength increased by 41-fold as compared to only gelatin hydrogel without compromising in biocompatibility (Jiang, Zhou, & Yang, 2018). Similarly, gelatin was used in a silk

fibrin—based hydrogel to prevent it from shrinking. Riboflavin was used as a photoinitiator to form hydrogel through dityrosine bonding. An increasing ratio of gelatin decreased the shrinkage of silk fibrin—based hydrogel and the width of the 3D-printed structure maintained its structural integrity (Lee, Shin, Shin, & Hyun, 2020).

Recently, methacrylated gelatin (GelMA) has been used as bioink in a number of studies for encapsulation of cells. It was first reported in 2000 by Etienne H. Schacht and coworkers and was named as gelatin methacrylamide. The addition of functional groups increases the mechanical strength of the hydrogel, which can be crosslinked by either a photo-radical initiation or by enzymatic crosslinking (Unagolla & Jayasuriya, 2020). Ye et al. used GelMA hydrogels for peripheral nerve regeneration using digital light processing—based 3D printing technology. Nerve guidance conduits of desired shape were printed, which supported the proliferation of nerve cells along the longitudinal channel (Ye, Li, & Yu, 2020).

A high concentration of GelMA is suitable for 3D printing with high printability characteristics; however, it decreases the cell viability. On the other side, a low concentration ($\leq 5\%$ w/v) is more suitable for cell encapsulation but has three disadvantages, such as (1) a low concentration of GelMA leads to low viscosity that affects the structural integrity of the printed structure, (2) the gelation time also becomes less at lower concentration, and (3) the initial mechanical strength of the printed structure is less to allow the scaffold to maintain its geometry. To overcome these hindrances, GelMA has been mixed with other polymers, such as alginate/gelatin (Chung, Naficy, & Yue, 2013), methacrylated hyaluronic acid (Skardal, Zhang, & McCoard, 2010), alginate (Colosi, Shin, & Manoharan, 2016), alginate/gelatin/hydroxyapatite (Wüst, Godla, Müller, & Hofmann, 2014), and gelatin (Yin, Yan, & Wang, 2018). Addition of gelatin was able to regulate the viscosity of GelMA bioink and provided an additional reversible thermo-crosslinking mechanism. The initial crosslinking was done by thermal and after printing was then stabilized by photo-crosslinking. This approach significantly improved the printing ability of GelMA hydrogel (Yin, Yan, & Wang, 2018). Another study used 1 w/v% 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-oxidized cellulose nanofibrils with low concentration of GelMA. The photo-crosslinking ability was enhanced and mechanical strength was improved in the range of 2.5–5 kPa. The printed scaffold was biocompatible in nature and promoted the proliferation of fibroblast cells (Xu, Molino, & Cheng, 2019).

Poly(ethylene glycol)-based hydrogels

PEG is one of the most commonly explored hydrogel among synthetic polymers ranging from a wide number of applications, from tissue engineering to ocular drug delivery systems. PEG is widely used for 3D cell culture systems due to its biocompatibility, nonimmunogenic, and easy to engineer its structure. It is a hydrophilic polymer with two hydroxyl groups on each end which can be substituted with other functional groups, such as amine, methoxyl, carboxyl, acetylene, and thiol groups. PEG hydrogels are crosslinked via photopolymerization that gives control over its temporal and spatial structure (Reid, Gibson, & Singh, 2015; Zhu, 2010). Some modifications such as PEG dimethacrylate, PEG diacrylate, and multiarm

PEG acrylates are not biodegradable in natural environment. Since PEG is a synthetic polymer, it is easy to control the chain lengths allowing the researcher to tune its mechanical property for different cell response. PEG can also be functionalized with different copolymers to form different biomaterials, for example, PEG-polybutylene terephthalate copolymers for growth of skin and bone cells.

3D printing has emerged as a promising technology for the printing of vascular network by controlled printing of cells and biomaterials. Most of the techniques follow a sacrificial method where a template bioink is first printed followed a second layer of ink. The template bioink is then removed to obtain hollow-like structure similar to blood vessels. These channels can then be seeded with endothelial cells for proper functioning of the printed structures (Miller, Stevens, & Yang, 2012). In a study by Skardal et al., bioprinted blood vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral PEG tetracrylates Thiolated hyaluronic acid was used to encapsulate NIH 3T3 cells by adding agarose microfilaments and crosslinking with tetrahedral PEG tetracrylates. The fabricated hydrogel showed significant high shear modulus with good cell viability (Skardal, Zhang, & Prestwich, 2010).

Another approach by Jia et al. developed a cell responsive bioink consisting of 4-arm poly(ethylene glycol)-tetra-acrylate (PEGTA), GelMA, and sodium alginate in a coaxial 3D printer where the vasculature structure was developed without the sacrificial ink. As depicted in Fig. 2.3, the coaxial printing gives an advantage of printing perfusable tubes of varying diameter. Crosslinking of the polymer blend was first done ionically by calcium ions followed by photo-crosslinking of GelMA and PEGTA. PEGTA improved the mechanical strength of the construct while other polymers provided biocompatibility to the structure to improve the proliferation of the cells (Jia, Gungor-Ozkerim, & Zhang, 2016).

Another study by Li et al. fabricated a bone scaffold using PEG, polylactic acid, ceramic (nanohydroxyapatite), and dexamethasone (drug to promote osteogenic differentiation). Since dexamethasone is reported to attenuate tissue response, a sustained release of the drug is required. 3D printing of the scaffold enables simultaneous blending with the matrix, which helps in sustained release of the drug without compromising the effectiveness of the drug (Li, Wang, & Wang, 2018). 3D printing offers faster processing of scaffolds with better control over microporous structure without the use of any specific tools or dies. It also helps in controlling the porosity of the structure. Bose et al. fabricated β tricalcium phosphate (TCP) scaffold with a coating of curcumin-poly(ϵ -caprolactone)-PEG. Improved osteogenic and angiogenic properties were observed with interconnected macro and micro pores (Bose, Sarkar, & Banerjee, 2018). The scaffold was able to mimic the highly porous nature of the bone.

Scaffold-free bioprinting

Spheroids

A bioink is generally defined as—“a formulation of cells suitable for processing by an automated bio fabrication technology that may also contain biologically active

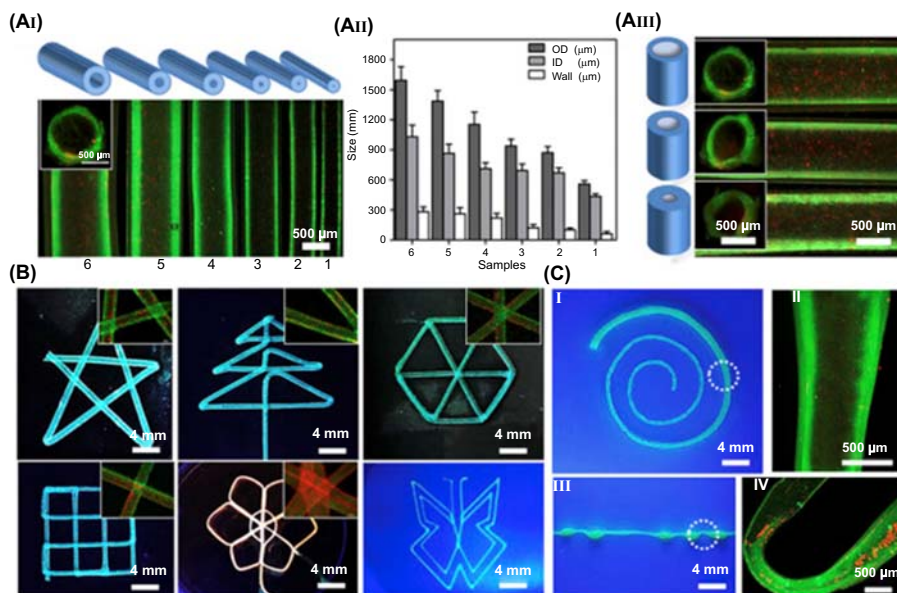


Figure 2.3 (A_I) Fluorescent images of 3D-printed tubes of different diameters. (A_{II}) Quantification of tubes with same diameter but varying different inner diameters. (A_{III}) Cross-sectional view. (B) Perfusable tubes of different shapes. (C) 3D printed perfusable tube with increasing diameter.

Source: Reproduced with permission from Jia, W., Gungor-Ozkerim, P.S., Zhang, Y.S., et al. (2016). Direct 3D bioprinting of perfusable vascular constructs using a blend bioink. *Biomaterials* 106:58–68.

components and biomaterials.” Bioink is generally considered a biomaterial that can form a scaffold for the support or encapsulation of cells; however, certain limitations are also associated with scaffold-based bioprinting. These include risk of infection/contamination, generation of immune response, and disease transmission (Nakayama, 2013). To overcome these problems, scaffold-free bioprinting has emerged, where a direct sheet of cells can be printed on a template and the group of printed cells is called as spheroids. Various cell types have been explored for spheroid constructs. 3D printing of spheroids also has certain limitations that need to be addressed to fully explore its potential. These limitations include—the structural integrity of spheroids is difficult to maintain and spheroid of different composition cannot be placed in arbitrary positions (Murata, Arai, & Nakayama, 2020).

In vitro spheroid cultures have been developed for applications such as drug screening, toxicity, efficacy, and disease progression; however, the cellular patterning structure is difficult to obtain in culture conditions. 3D printing allows spatial control over cell patterning. Pancreatic cell spheroids were printed using laser-assisted bioprinting technique for studying pancreatic ductal adenocarcinoma progression, a common malignancy of the pancreas. The 3D-printed spheroids had both acinar and ductal cells, which mimicked the initial stages of the disease

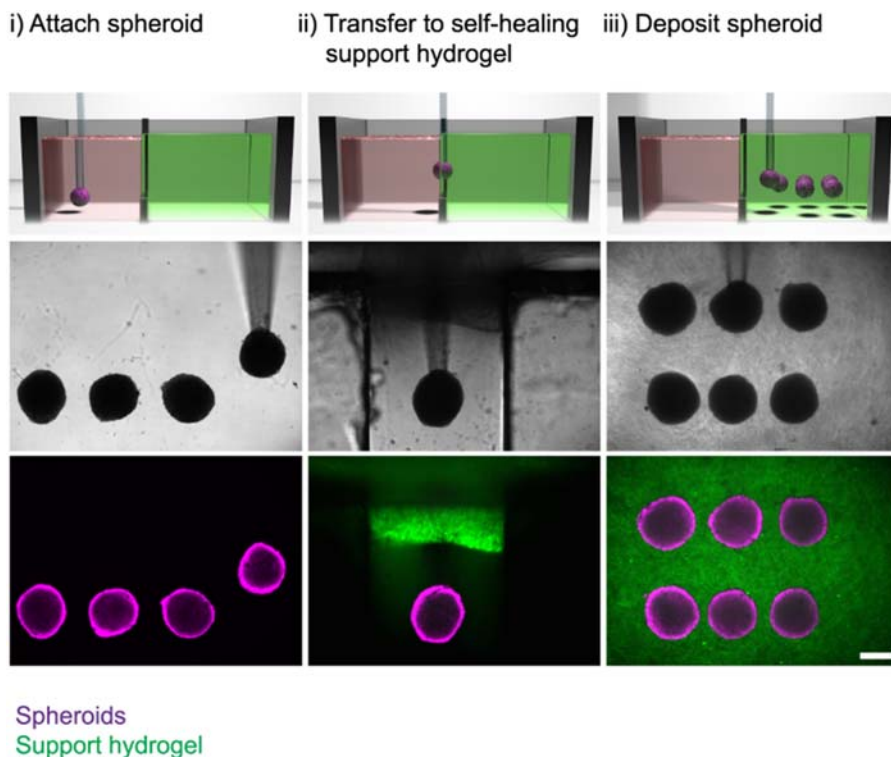


Figure 2.4 Schematic, brightfield and fluorescent images of mesenchymal stem cell spheroid: (i) a spheroid cultured in media is vacuum aspirated (ii) transfer of the spheroid to a support hydrogel that self-heals due to its shear thinning property (iii) deposition of the spheroid at the desired location

Source: Reproduced with permission from Daly, A. C., Davidson, M. D., Burdick, J.A. (2021). 3D bioprinting of high cell-density heterogeneous tissue models through spheroid fusion within self-healing hydrogels. *Nature Communication* 12:1–13.

progression. 3D printing has been widely used for developing disease models and thus for therapeutic applications (Hakobyan, Médina, & Dusserre, 2020). Daly et al. used bioprinting approach to transfer spheroids into self-healing support hydrogels where it maintained its structural integrity as depicted in Fig. 2.4. The printed structure was able to retain its patterning and can be fused together into high cell density microtissue structure. The bioprinting involved three major steps: (i) A mesenchymal stem cell spheroid cultured in media is vacuum aspirated, (ii) transfer of the spheroid to a support hydrogel that self-heals due to its shear thinning property, (iii) deposition of the spheroid at the desired location. These spheroids can be fused together to obtain the desired patterning structure (Daly, Davidson, & Burdick, 2021).

Self-healable hydrogel was also reported by Zhang et al., for bioprinting of scaffold for spheroid growth and development. A mixture of chitosan methacrylate and

polyvinyl alcohol was used as the hydrogel mixture that can be photo-crosslinked by UV light. The fabricated hydrogel had shear thinning property, gel–sol transitions and good yield strength—requirements for extrusion based bioprinting (Zhang, Cong, & Osi, 2020). Aguilar et al. reported a “Kenzan method” of scaffold-free printing where an array of stainless steel needles was used in a predetermined manner. This method uses natural cell-cell adhesion proteins to fuse the spheroids together. As the cells come in close contact, proteins such as integrins, connexins, or cadherin-mediated adhesion comes into play and ECM develops around it to provide the structural integrity of the printed geometry (Ahmad, Lee, & Shin, 2017). The major limitation in such a method is the size of the spheroid. The optimum size is 500 μm , if the size is significantly larger, the larger spheroids tend to push the below spheroid, thus rupturing of the spheroid may happen as the printing is from bottom to top, also oversized spheroids may distort the spacing of the printed structure; if the size is significantly lower the spheroids may not interact with each other, which is necessary for cell-cell interaction and building up of ECM and the spheroids may split as they are placed over the Kenzan needles (Aguilar, Olivos, & Brinker, 2019).

Decellularized matrix

The ECM is derived from any target organ or tissues where the cells have been removed to obtain the natural vascular structure. The process of removing the cell debris is known as decellularization. The main advantage of decellularized ECM is that the material does not contain any immunogenic components while retaining the natural environment of the cells—such as proteins, glycosaminoglycans, proteoglycans and growth factors. A tissue-specific ECM provides the microenvironment for cell differentiation, adhesion, mechano-transduction and remodeling ability (Heath, 2019; Keane, Swinehart, & Badylak, 2015).

As the techniques for decellularization and recellularization have emerged, hydrogels from different decellularized tissue types have been reported, such as heart, bone, tissue, liver, lungs, and urinary bladder. These hydrogels are being explored for tissue growth and development (Garreta, Oria, & Tarantino, 2017). Recently, decellularized ECM have been explored as a bioink for 3D bioprinting technology; however, it is still in early stage of research and needs to be optimized and engineered to overcome its limitations. The major limitation is the low viscosity, which compromises with the fidelity of the printed structure. Few studies have been published that make use of decellularized material as a bioink for 3D printing application and needs to be engineered to explore its potential.

Pati et al. were the first to report the use of decellularized ECM as a bioink for 3D bioprinting. They have used polycaprolactone as a support material, which was co-deposited with decellularized ECM derived from different tissues such as heart, cartilage, and adipose porcine tissue (Pati, Jang, & Ha, 2014). They also reported the development of skeletal muscle-derived decellularized ECM hydrogels for 3D printing of muscle structures with polycaprolactone as a support material

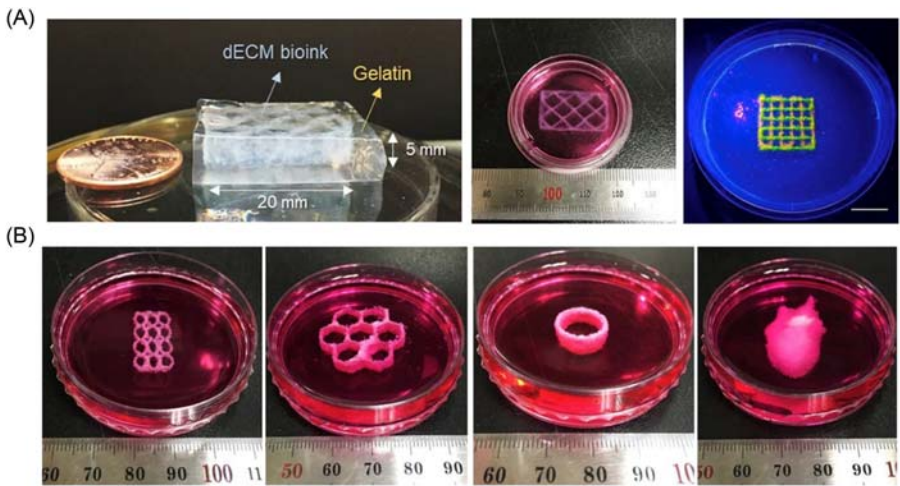


Figure 2.5 (A) 3D printing of muscle-derived dECM on a granule-based reservoir; removal of the printed structure and growth in a culture medium; fluorescent images indicate the possibility of printing heterogeneous tissues using different cells encapsulated in bioink. (B) 3D bioprinting of different complex structures and maintaining their shape fidelity. *Source:* Reproduced with permission from Choi, Y.-J., Jun, Y.-J., Kim, D. Y., et al. (2019). A 3D cell printed muscle construct with tissue-derived bioink for the treatment of volumetric muscle loss. *Biomaterials* 206:160–169.

(Choi, Kim, & Jeong, 2016). Their study also demonstrated the use of gelatin as support material. As depicted in Fig. 2.5, decellularized ECM (dECM) from muscle tissues were printed on a granular structure using coaxial printing with muscle and vascular dECM bioinks (Choi, Jun, & Kim, 2019).

Gelatin-based hydrogels were also used by Skardal et al. for preparing dECM-based bioink. Gelatin mixed with hyaluronic acid was used to prepare modular hydrogels, which were supplemented with ECM solutions of skeletal muscle, cardiac, and liver tissues. The authors used a two-step crosslinking method to print geometries of varying stiffness ranging from 100 Pa to 20 kPa. A broad range of mechanical stiffness allows to biomimic different tissues in the body with different functionalities (Skardal, Devarasetty, & Kang, 2015). Another study by Jang et al. used pig heart dECM to fabricate a hydrogel, which was combined with progenitor cells of human cardiac tissue. They have also used two-step crosslinking process—thermal and vitamin B2 induced UVA crosslinking methods. The biomechanical properties of the printed structure were similar to that of native cardiac tissue.

Conclusion

3D bioprinting has emerged as a potential tool in revolutionizing medical field mainly—tissue engineering, organ replacement, drug delivery, drug screening, and

toxicology models. 3D bioprinting technology is still in the research phase and needs to be explored before it can come to product and prototype level. There have been reports of nozzle designing and modifications in the printing types, similarly, bioinks have been engineered to fit the biomechanical properties of different tissue constructs. New biomaterials need to be explored that can fit to different parameters of a bioink—biocompatibility, structural integrity, degradability, and mechanical strength for long-term structural complexes that can mimic the structural and vascular complexity of natural human tissues or organs. As a rapidly developing field of research, advancements in bioink can help in further development of medical-based healthcare outcomes for patients and for society as a whole.

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Three-dimensional printing of live cells, tissues, and organs

3

Chapter outline

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Introduction

With advancements in technology, new directions are emerging for betterment of human. Three-dimensional (3D) printing is one such advancement in technology that has found applications in every sector of society for the welfare of mankind. In the field of agriculture, it is helping in the development of a better farming system by modifying the traditional equipment and tools used in farming. This technique is used efficiently by farmers in Myanmar. Earlier they were using the slow and expensive computer numerical control machines; however, with the advent of 3D printing technology, they developed quality tools that reflect improvements in their manufacturing process. Similarly, in the food sector, this technology is helpful in developing new attractive and designer food products, which are in high demand in the market. This technology is proving itself in every sector of society; however, the most appreciable use of 3D printing is in the medical/pharma sector, which is directly related to human health. In this area, the technology is emerging as a boon for the cure of humans for various simple to life-threatening diseases.

3D printing is based on the concept of additive manufacturing, in which materials are laid down layer by layer according to the computational design, giving rise to the

desired structure. The required computational designs may be created with the help of a number of available software like computer-aided design (CAD) or computed tomography (CT), etc. 3D bioprinting, which is 3D printing using biological material, is a unique method of biofabrication. This technology can generate 3D products by using a variety of materials/biomaterials. In 2015, Aprecia Pharmaceuticals, a US-based company, produced the first tablet manufactured using 3D printing, which is under process for FDA approval. In the present chapter, we discuss how this technology is affecting medical science and up to what level people would be benefitted by it.

Components of three-dimensional printing

To perform printing of any biological material in 3D, two main components are required, namely a biological component and the nonbiological component. Nonbiological components consist of hardware, software, and controlling parameters, whereas biological components consist of bioink. The features and designs of 3D printer have been discussed in detail in Chapter 1; however, here we briefly discuss the characteristics of bioink only.

Bioink and its role in three-dimensional printing

Bioink is one of the prime requirements of 3D printing. This term was first used in 2003 in reference to organ printing and the terms bioink and biopaper were introduced together (Groll et al., 2018). Initially, this concept was used to print a cell and the combination of hydrogel with live cells was called bioink. Thus, one can say that the bioink is the cellular material that is positioned in any 3D matrix. It is a combination of appropriate biomaterials that mimic the environment in the form of the extracellular matrix, which is helpful in the adhesion, proliferation, and differentiation of living cells within the environment. The components which are used in the preparation of bioink are generally prone to crosslink and settle immediately after printing. This tendency of bioink helped in providing the final shape of the desired output. Every material is not suitable to make bioink for 3D printing and only certain types of biomaterials can be used in the development of the bioink. These criteria sometimes create hurdles in the development of the required type of bioink, hence restricting the application of 3D printing in certain areas. With the rising interest in the field of 3D bioprinting, researchers focused their studies on the development of new materials for bioink, which, in turn, will give new directions to 3D printing. Natural, synthetic, or combination of both types of materials are used as bioinks.

Characteristics of bioink that makes it special

Certain basic parameters/characteristics are essential to be present in biomaterials for them to be categorized as bioink, such as an ideal bioink should possess desired mechanical, rheological, chemical, and biological properties.

Bioinks containing their proper physicochemical properties can lead to the printing of output with the desired shape, biocompatibility, and biodegradability (Singh, Bandyopadhyay, & Mandal, 2019). Among the various properties of bioinks, biological properties refer to the biocompatibility and biodegradability of the developed print in the host environment where they are implanted. Biocompatibility feature gives a tendency to printed objects (print) so that they can easily adjust themselves in the foreign body and without making any accumulation, which may be harmful to the host body, whereas the biodegradability feature enables printing, such that after a certain time they easily degrade or excrete out from the body. Physicochemical properties provide the ability to the print to make chemical modifications if required. It is not easy to predict the chemical responses of the body during the tenure of the print inside the body, therefore, print should have the ability to meet such types of changes. Print temperatures that do not exceed physiological temperatures, mild cross-linking, or gelation conditions. Mechanical properties provide mechanical strength and durability to the bioactive components that are nontoxic and should be modifiable by cells after printing as and when required in the host environment. The rheological properties of the bioink depend on the viscosity of a bioink, which is essential for providing support for a particular cell type. For ideal printing conditions, the viscosity of the cells and the bioink material should be similar or close to each other, otherwise cells are considered to be under a stress condition while printing or printability is compromised (Singh et al., 2019).

In addition to these characteristics, according to the requirement of bioink, the preparation process should be easy and cost-effective so that it can be produced in large quantities. At this time, the material of bioink has the potential to be cultivated on a large-scale with the least batch-to-batch variations. Different bioink materials have different characteristics and according to the requirement, we select the suitable material for the formulation of bioink. Alginate is calcium, magnesium, and sodium salts of alginic acid present in the cell walls of brown algae. It is one of the most commonly used natural materials used in the formulation of hydrogel for drug delivery. Cross-linking of alginate with divalent ions (like Ca^{2+}) improves the stability of prints. It is highly biocompatible as its physical properties can be tailored according to the need for cellular growth or differentiation in both in vitro and in vivo conditions. Freeman and Kelly (2017) used this hydrogel for mesenchymal stem cells (MSCs) differentiation in tissue engineering. Agarose is a natural polysaccharide with repeated units of agarobiose and is generally extracted from red seaweed. The low gelling temperature (32°C), biocompatibility, and mechanical strength of agarose make it a suitable material to be used as bioink. Due to its high gelling property, it does not require an additional cross-linker in the reaction and makes the printed structure more feasible. Several reports mention the use of agarose-based hydrogels for tissue engineering of cartilage (López-Marcial et al., 2018). Some commonly used natural and synthetic materials for bioinks along with their specific features have been summarized in Table 3.1 and Fig. 3.1. The properties of various natural and synthetic biomaterials are discussed in detail in Chapter 2.

If we compare the performance of natural and synthetic bioink for printing, natural materials due to their high biocompatibility are preferred over synthetic materials. However, there are still some big challenges for natural materials to be considered an

Table 3.1 List of Natural and synthetic materials used as bioink in bioprinting.

Natural	Source	Reference
Agarose	Polysaccharide (Polymers of sugars) isolated from red seaweed	Maciel et al. (2008)
Alginate	Polymers of biological compounds isolated from brown algae	Aprilliza (2017)
Chitosan	Polysaccharide isolated from the exoskeleton of shrimp and other shellfish. Chitosan derived from non-animal-derived sources can be isolated from fungal fermentation.	Sastry, Shrivastava, and Venkateshwarlu (2015)
Collagen	Structural protein present in skin and connective tissues	Bay-Jensen et al. (2013)
Decellularized ECM	Extracellular matrix tissue material isolated from inhabiting native cells	Yao et al. (2019)
Fibrin/fibrinogen	Insoluble protein developed during the process of blood clotting	Vilar, Fish, Casini, and Neerman-Arbez. (2020)
Hydroxyapatite	Naturally occurring mineral found in the teeth and bones as calcium apatite	Pokhrel. (2018)
Hyaluronic acid (HA)	Glycosaminoglycan (non-sulfated) widely distributed throughout the various tissues like epithelial, connective, and neural.	Pirrello et al. (2019)
Gelatin	Protein substance produced by the partial hydrolysis of collagen.	Singh et al. (2019)
Synthetic Graphene	Carbon-based material, which could be viewed as graphite sheet of one atom thickness	Komeily-Nia et al. (2020)
PCL/PLA/PLGA	Biodegradable, polymers/copolymers having thermoplastic nature	Xia, Shi, He, Pan, and Liu. (2018)
Pluronic F127	Block copolymer of poly(ethylene oxide) and poly(propylene oxide)	Zhang, Metzger, Hackel, Bates, and Lodge. (2020)
PEG	Phase change material formed by the reaction of ethylene oxide with water	Yang, Pang, Liu, and Guo. (2018)

ideal bioink. Some of them are low mechanical strength, structural stability, shape fidelity, etc., which directly affect the printability of the constructs (Serna et al., 2019; Chimene, Lennox, Kaunas, & Gaharwar, 2016). Scientists are still working to find out ways to overcome these issues and reporting their ideas, for example, Gungor-Ozkerim, Inci, Zhang, Khademhosseini, & Dokmeci (2018) suggested the use of easily available and cheap synthetic polymers like polyethylene glycol (PEG), polylactic acid (PLA), or polycaprolactone (PCL), etc. along with the natural material to increase the mechanical strength of the blend. Moreover, this mixing of synthetic material with

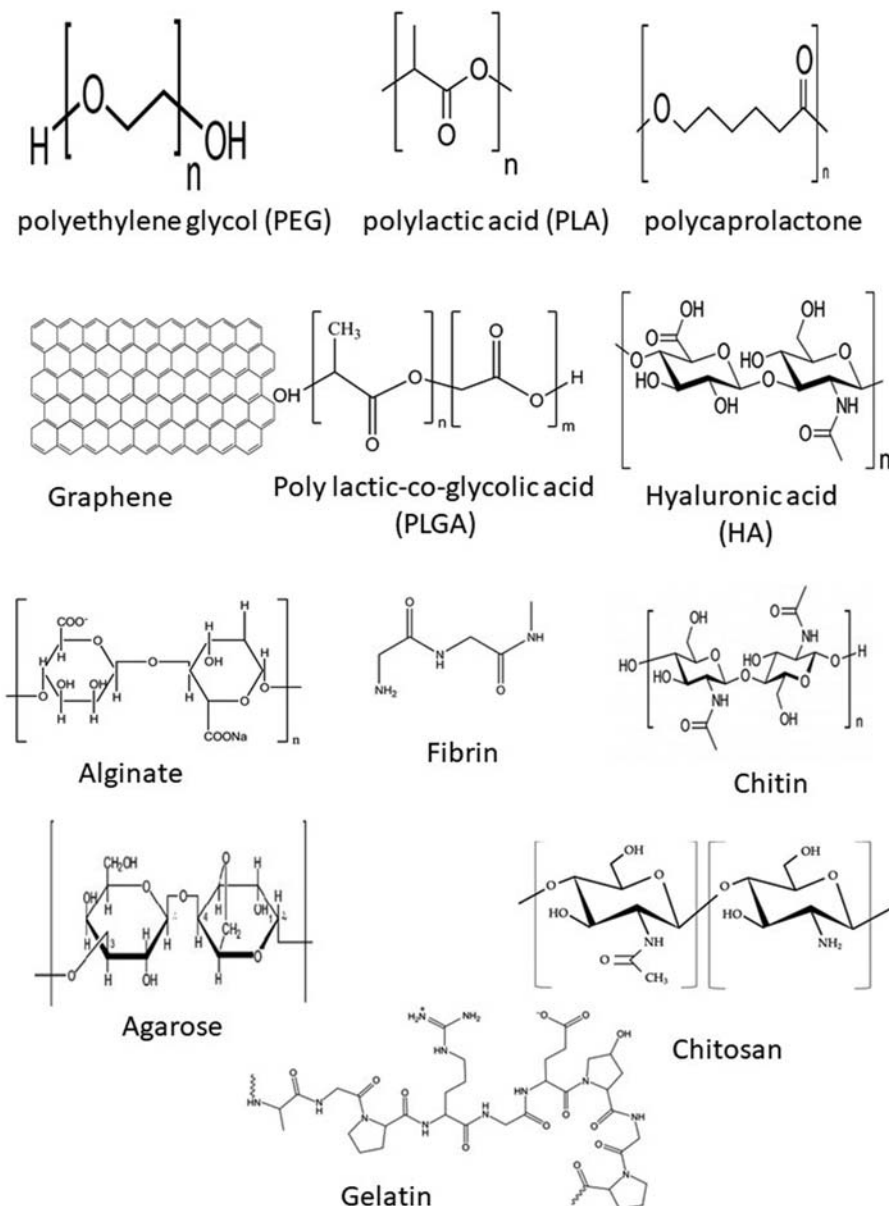


Figure 3.1 Chemical structures of different materials used in the development of bioinks.

natural ones decreases the degradation rate of the construct. However, this mixing may affect the biocompatibility of the construct and therefore the selection of materials and concentration of mixing is the prime area of research. This study comes under the bioink formulation and optimization is one of the prime focus of the research.

Three-dimensional bioprinting of live cells

3D printing technology offers high efficiency in the desired output, is capable of producing single products, and the instrument can be easily modified as per requirement. Due to these basic characteristics, this technology is gaining more attention in diverse fields globally. When this technology is applied in the biological field to print live cells, tissues, or even organs, it is termed as bioprinting, including cell printing, tissue printing, or organ printing. Bioprinting is a promising technology in the area of tissue engineering wherein computer-controlled deposition of cells or biomaterial is performed layer by layer, which results in the desired output.

Faulkner-Jones et al. (2015) in their work developed an advanced cell printing platform. This system has four dispensing systems of nanoliter capacity made of a solenoid valve. The nozzle size that was selected for their system had an internal diameter of $101.6\ \mu\text{m}$. These dispensing systems were attached through flexible tubing to the reservoirs (under static pressure) containing the desired bioink. The dispensing system and the reservoir are mounted on the head of an enclosed chamber of 3D printer. This modification reduces the overall weight and size of the instrument, which makes it suitable to be kept in a laminar hood for providing sterile conditions, which are the prime requirements for printing of any live cells. A schematic representation of the process and the instruments involved in 3D bioprinting are summarized in Fig. 3.2.

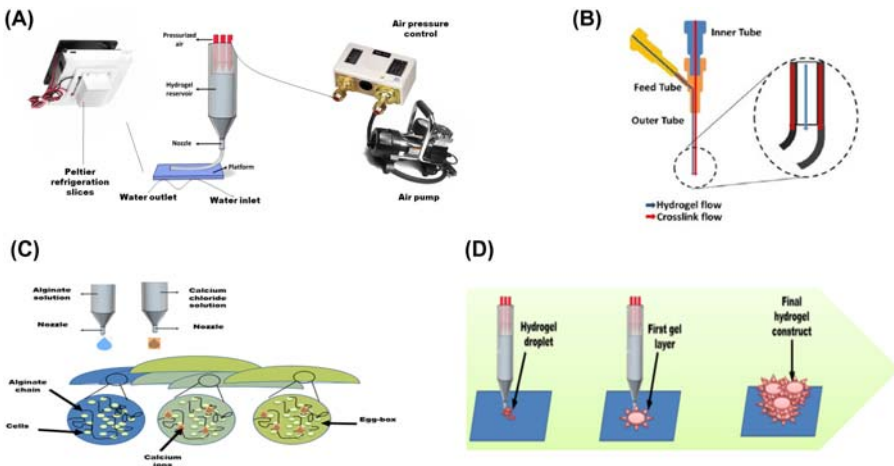


Figure 3.2 Schematic representation of 3D bioprinting and process (A) basic instrumentation of 3D bioprinting, (B) needle design, (C) hydrogel printing process using live cells and alginate solution and (D) layer-by-layer deposition of cells or biomaterial resulting in the designed output.

Cell sources and bioink preparation

As per the requirements, various cell lines can be used as bioink for printing purposes. Faulkner-Jones et al. (2015) reported the successful use of human-induced pluripotent stem (hiPS) cell lines (RCi-22 and RCi-50) and human embryonic stem cell (hESC) lines (RC-6 and RC-10). The hiPS cells are an example of pluripotent stem cells that have the ability to be generated through somatic cells, whereas hESC are developed from the inner cell mass of embryos at the stage of supernumerary blastocysts. Other cell lines that are reported to be used in the bioprinting purpose are mouse fibroblasts cell line L929 (He et al., 2016), rat heart endothelial cell lines (RHECs) (Khalil and Sun, 2009), etc.

For the preparation of cell-laden hydrogel ink, cell lines should be introduced under suitable conditions. Cell confluency is the basic thing that should be considered while such type of preparation and it should be nearly 95%. The cells were incubated in a CO₂ incubator at 36.0°C–37.5°C, 5.0% ± 0.5% CO₂. The centrifuged cell mass of the culture media is used to resuspend in the selected cell culture media like MEM with basic growth factors to a suitable concentration (2×10^6 cells/mL).

The hydrogel is prepared separately by using any suitable natural or synthetic polymers, which are already discussed in the above section of this chapter. The cells and the hydrogel can be mixed in an equal ratio by stirring the material for 5–10 minutes at 37°C using a magnetic stirrer. The interlinking between the selected polymer is an essential criterion for any hydrogel and it depends on the number of polymer chains and multivalent cations (e.g., Ca²⁺, Ba²⁺, Zn²⁺, Cu²⁺) used in the preparation under the experimental conditions (Slaughter, Khurshid, Fisher, Khademhosseini, & Peppas, 2009). These cations alter the stiffness of the material and make the material useful. When the paramagnetic metal ions are used in the formation of hydrogel then the developed hydrogel is known as ionotropic hydrogels (Winkleman, Bracher, Gitlin, & Whitesides, 2007). Multivalent cations of alkaline earth metals, transition metals, and lanthanide metals can form cross-linking alginate acids.

The most commonly used cations are as follows:

1. Alkaline earth metals, such as Ca²⁺, Sr²⁺, Ba²⁺
2. Transition metals, such as Pb²⁺, Cu²⁺, Cd²⁺, Zn²⁺, Ni²⁺, Al³⁺, Fe³⁺, Sn⁴⁺
3. Lanthanide metals, such as La³⁺, Nd³⁺, Eu³⁺

Khoury, Slawinski, Collison, and Popa (2020) reported that using Zn²⁺ and Cu²⁺ ions can change the stiffness of the BSA hydrogel and cast it in the form of spring shape and another flower-cast material was used in the formation of a ring shape successfully. They also discussed the major advantages of the protein hydrogels, which are programmed by the cations: (1) higher stiffness than the regular buffer, (2) ability to be converted into complex designs, (3) rapid diffusion of these ions leading to irreversible morphing.

Cell printing

The developed hydrogel is a type of biomaterial used for printing of the desired output. With developments in the field of 3D bioprinting, the methods used for printing have also improved according to the need. Cellular inkjet printing (Xu, Chai,

Huang, & Markwald, 2012; Matsusaki, Sakaue, Kadowaki, & Akashi, 2013; Ferris, Gilmore, Beirne, McCallum, & Wallace, 2013), extrusion-based printing (Huang, Qu, Liu, & Chen, 2014; Chung et al., 2013; Ozbolat & Hospodiuk, 2016), laser-assisted bioprinting (Zhang, Xiong, Mei, Huang, & Chrisey, 2015; Xiong, Zhang, Chai, Huang, & Chrisey, 2015), and stereolithography (Arcaute, Mann, & Wicker, 2010; Huang et al., 2014) are some of the most common printing methods used by researchers worldwide. Different types of printings that are used by researchers for the development of different objects are summarized in [Table 3.2](#).

Cellular inkjet printing

It is the oldest form of printing that is used. Cell and other required biomaterial are used as bioink for printing. Based on the printing, inkjet printing can be of two types, namely continuous inkjet (CIJ) printing and drop on-demand (DOD) inkjet printing (Ozler, Kucukgul, & Koc, 2015). In the CIJ method, a force is applied on the liquid bioink to pass through an orifice under experimental external pressure, which converts the bioink in the form of a stream of droplets and placed on according to the predesigned framework whereas in DOD a pressure is applied on the bioink only when it is required. Furthermore, based on the approach, inkjet can be thermal or piezoelectric. In thermal inkjet, a microheater is used to vaporize the fluid (bioink) to generate the pulse, which comes in the form of a droplet from the nozzle, whereas in the piezoelectric, the piezoelectric actuator is used to create shockwaves (mechanical pulse) that expel the bioink from the nozzle.

Extrusion-based bioprinting

It uses self-assembling property of cells to construct predesigned biological construct. The basic principle of this technique is to provide a continuous flow of biomaterial in the form of filaments along with hydrogel, copolymers, and living cells through the nozzle according to computer-designed structure. CAM-CAD software, which is used for designing and directing printers to deposit the material in a layer-by-layer manner to develop a whole construct. Using extrusion-based printing, “Scaffold-free” bioprinting has also been developed, which is based on the principles of liquidity of tissue and tissue fusion of multicellular components.

An ideal **laser-assisted bioprinter** generally contains three basic elements, that is, (1) the pulse laser source, (2) the ribbon, and (3) the substrate which is received. The ribbon is a multilayer component of this setup, which is composed of transparent support and a layer of transfer bioink. Because of the transparency of the support, laser radiation can easily penetrate through it. This support is further coated with a bioink, which has to be transferred during the process. The bioink contains a heat-sensitive biological material according to the need of the process. Based on the optical properties of the wavelength of the laser beam or the bioink, a laser-absorbing interlayer is placed between the layer of support and bioink. This interlayer is generally made up of thin-film (nm) of metal (Au, Ti, Ag), metal oxide (TiO₂), or photo-decomposing volatile polymer (triazene).

Table 3.2 Different types of printing used by the researchers.

	Printing type	Cell printed	References
Cellular inkjet printing	—	Individual living cells are seeded.	Nakamura et al. (2005)
	Inkjet	Printing of hybrid cell and proteins are done.	Derby (2008)
	Scaffold-free inkjet	Zigzag cellular tubes are printed in 3D form.	Xu et al. (2012)
	Automatic and rapid inkjet	Human tissue chips (3D).	Matsusaki et al. (2013)
Extrusion-based printing	Drop-on-demand jetting	Alginate microparticles are printed (with a defined shape).	Gao, et al. (2016)
	On-demand printing	Printing of living cells and tissues.	Ferris et al. (2013)
	Bio-ink properties with printability	Living cells and tissues are printed.	Chung et al. (2013)
	Biological laser printing	Printing of 3D cellular structures.	Barron et al. (2004)
	High-throughput laser printing	Printing of biomaterials and cells for tissue engineering.	Guillemot et al. (2010)
	Laser printing of alginate (viscoelastic) solutions	Jetting dynamics were studied using time-resolved imaging technique.	Zhang et al. (2015)
	Freeform drop-on-demand laser printing	Printing of 3D cellular and alginate constructs.	Xiong et al. (2015)
Laser-assisted bioprinting	Photopolymerizable hydrogels	Constructs of tissue-engineering were rapidly prototyped.	Dhariwala et al. (2004)
	Bioactive PEG (polyethylene glycol)	Multi-material scaffolds were spatially controlled.	Arcaute et al. (2010)
Stereolithography		Biomimetic microstructures (3D) were printed for cancer cell migration. Biomed.	Huang et al. (2014)

Stereolithography printing

It is another technique that is based on the principle of polymerization of light-sensitive polymers by controlled light glinted from digital micromirrors used in the setup. By applying these techniques, the fabricated structure exhibited high cell viability, proliferation, and metabolic activity.

Every method has its advantages and disadvantages, such as the **inkjet printing** process has high fabrication speed, low production cost, a wide range of availability, and the ability to print low-viscosity biomaterials; however, there is always a risk for cell damage due to thermal or mechanical stress, inability to provide a continuous flow, poor results for vertical designs, and the results of the cell encapsulation process also vary from batch to batch. Likewise, *extrusion-based* bioprinting is a common method that is used to deposit very high cell densities in the required shape and is capable of printing a range of biomaterials; however, the pressure used during the process may affect the cell viability and it is only applicable for viscous bioinks. *Laser-assisted bioprinting* having fine resolution represents a special method; however, the high cost of printing systems and lack of commercial 3D laser bioprinters are some the major challenges that limit widespread usage. Similarly, high accuracy and cell viability in printing are reported in a nozzle-free technique. Stereolithography is a technique wherein printing is time independent of complexity; however, the lack of printing multi-cells and the damage of cells during photocuring are the points of concern (Derakhshanfar et al., 2018).

Three-dimensional bioprinting of tissues

The tissue is the collection of cells in a definite pattern to give its specific characteristics. With the help of various designed 3D printers, controlled deposition of cells on computer-designed pattern gives rise to the tissue in the 3D stage. This bio-printed tissue has a lower risk of failure during the treatment of grafting in comparison to traditional. With the advancement of technology, to date, a number of tissues are printed and scientists are studying the characteristics and viability of the printed tissues, such as ears, skins, bones, vascular tissues, and cartilaginous structures (Papaioannou et al., 2019; Wang et al., 2019).

Mainly, two types of tissue engineering methods are known, one is the traditional “top-down” and the other is the “bottom-up” approach and their steps are summarized in Fig. 3.3.

In the “top-down” approach, live cells are seeded either on the scaffold or entrapped within the porous scaffold to form the tissue construct in 3D geometry. This traditional approach has many limitations, such as when there are multiple types of cells then their *precise positioning* is a big challenge. Similarly, to attain *specific geometry and cell density* of the designed tissue is another question facing the researchers. Likewise, slow vascularization throughout the 3D space of the tissue construct and *diffusion limitations* are the points which have kept the

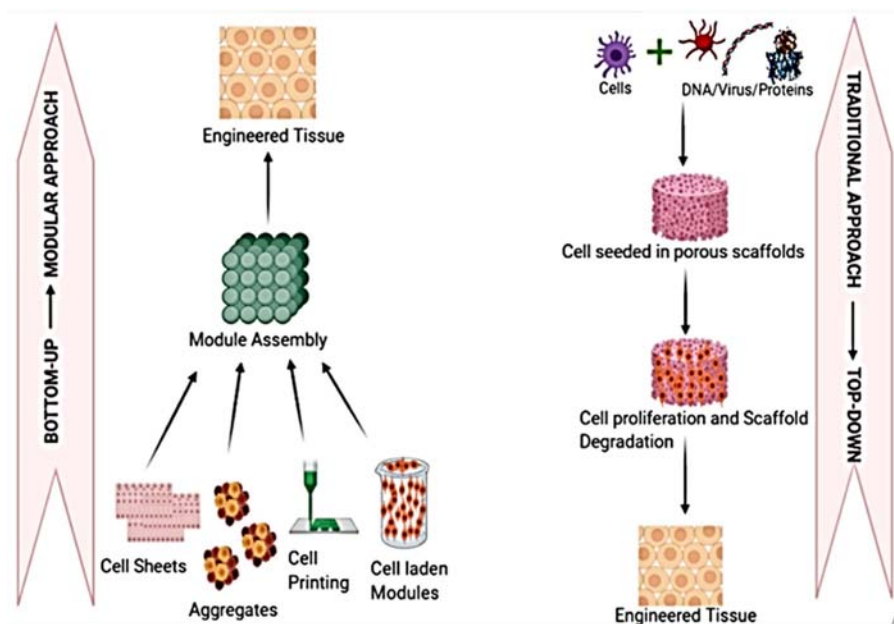


Figure 3.3 Two approaches: bottom-up and top-down used for tissue engineering.

researchers still struggling. To overcome these limitations, an alternative method of the bottom-up approach of tissue engineering is developed. In contrast to the top-down approach, the bottom-up approach contains the building blocks of the tissue structure like cells, other materials, and biological factors. These components are assembled giving rise to a large and complex structure of the tissue. It is comparatively easy to engineer the individual compartment (block) in the desired shape and size using the required type of cells and other materials and then assemble it into tissue-like architecture during scale-up. This process provides more control over the 3D arrangement of cells, biomaterial, or bioactive factors to give a more accurate structure to mimic the functions of the parent tissues.

Microgels, due to their adjustable physical properties, cell adhesivity, and biodegradability, are generally used as the building blocks in the bottom-up approach. Till date, various developments have been made using this microgel, such as *cell-laden microgels* wherein cells are *randomly assembled* into microvascular-like constructs, cell-encapsulated microgels containing defined shapes and sizes for self-assembly, and cell-containing microgels directly assembled for use of the microfluidic channel.

The cell-laden microgels developed from random assembly approaches can be used easily for developing big structures. However, this approach generally lacks control over the spatial arrangement of the microgels. On the other hand, the cell-laden microgel developed by the *manual assembling process* shows control over the spatial distribution of various cells. However, the slow process speed is

the challenging factor in this process. As an example, *cell-laden* microgel architecture developed through microfluidic device has high precision but the required instrument and the process is very complex and exhaustive. (Angelats Lobo, Ginestra, 2019), whereas direct assembly offers easy control over the architecture of the microgel but the process required organic solvents, which are generally cytotoxic to the cells, therefore, limiting the use of this technology to living cells. (Vukicevic, Mosadegh, Min, & Little, 2017). The use of acoustic waves is another alternative for a fast and noninvasive method for developing microgels, but this method also suffers from the limitation of precision in architecture.

Various cell lines used for the construction of specific tissues are summarized in Table 3.3.

Steps of three-dimensional printing technology for tissue engineering

There are different operating principles that are used in various types of 3D printing according to the desired output. Some of the established operating technologies are shown in Fig. 3.4. The fundamental steps used to generate the geometry are as follows: the initial step is the designing of the geometry of the output produced using CAD/CAM software. After designing, the prepared file format is converted into STL file format. The next important step is the selection of raw material according to the need then the designed geometry is printed layer by layer using a 3D printer of the required operating principles. The last step involves postprocessing according to the methods opted for 3D printing (Gupta, Bissoyi, & Bit, 2018).

Bottlenecks of three-dimensional printing for tissue engineering

The most crucial bottleneck that the current status of techniques related to bioprinting of the tissue is facing is the maintenance of cell viability within the bioprinted tissue (Gu et al., 2015). As we move from cell to tissue, structural complexity increases, which creates hurdles in the 3D printing process (Colosi et al., 2016). Increased complexity in design/structure also complicates the process of bioink development, such as the selection of raw material according to the design of the tissue (Vukicevic et al., 2017). In addition, the viscosity of the developed bioink is also a crucial point of the whole process, which reflects the success of the process (Zhang et al., 2017). This is because the developed bioink with desired viscosity and rheology should be strongly biocompatible to maintain the balance between the viability and functionality of the product after bioprinting (Angelats Lobo, Ginestra, 2019). To overcome the abovementioned limitations, a robust strategy is required that should be simple and quick on the one hand and on the other hand it also assures the cell viability and desired behavior.

Table 3.3 Bioprinting used in the field of tissue engineering.

Tissues	Cell line (s) used	Materials used	Applications	References
Adipocytes	BAP and WAP 1	Gelatin-based hydrogels (methacrylated)	The metabolic study was focused on to differentiate the brown and white adipose tissues.	Kuss et al. (2017)
	HUVEC 4 and HWA 3	PEG, Gelatin (methacrylated), and Hyaluronic acid (methacrylated)	4 A 5 Robust cryogel for adipose tissue engineering	Qi et al. (2018)
Myocytes	Schwann cells and L8 myoblasts	DMEM 1 and Alginate hydrogel	The study characterized the proliferative ability and cell damage occurring while and after bioprinting.	Ning et al. (2018)
	C2C12 myoblasts of Mouse	Gelatin/glycerol/fibrinogen/hyaluronic acid hydrogel	study the cell damage	Kang et al. (2016)
Skin tissues	Human mesenchymal stem cells and L929 fibroblasts	Calcium chloride and Methacrylated alginate hydrogel/gelatin hydrogel	The study described about bone tissue engineering using the MFT (Mesoscopic Fluorescence Tomography).	Tang et al. (2018)
	NIH/3T3 cell line	EGF and Methacrylated gelatin hydrogel	The study provided an insight for the application of regenerative medicine in tympanic membrane perforations.	Kuo et al. (2018)
	HaCat 8, HDF, and HEM 7	Photoinitiator (and tyrosinase), Collagen, and Methacrylamide hydrogel	Living skin constructs were printed and thus, were reported in the study.	Shi et al. (2018)

(Continued)

Table 3.3 (Continued)

Tissues	Cell line (s) used	Materials used	Applications	References
Hepatocytes	HUH7 1 and Small cholangiocytes of immortalized mouse	Pluronic F 127 (Sacrificial material) and dECM 2 (of the liver)	The study reported about the 3D bioprinting of liver tissues	Lewis, Yan, Su, and Shah. (2019)
Bone and cartilage	Primary chondrocytes and Other cells	Calcium chloride, PCL, and Alginate hydrogel	The study emphasized on the engineering of cartilage tissue.	You et al. (2016)
	Cartilage derived progenitor cells, Mesenchymal stem cells, and Primary chondrocytes	Gelatin hydrogel (methacrylated) along with a photoinitiator	The work focused on cartilage tissue engineering using reversible cross-linking strategies.	Gu, Tomaskovic-Crook, Wallace, and Crook (2018)
Cardiomyocytes	SVFC 4	hydroxyapatite hydrogel/PCL	The work was highly emphasized on 3D printed bone constructs and their prevascularization.	Kuss et al. (2017)
	hCPCs 2	cardiac ECM 3 and Gelatin hydrogel (methacrylated)	The work was found to be associated with 3D-printing of Cardiac (heart) patches.	Bejleri et al. (2018)
	Human coronary artery endothelial tissues	Calcium chloride/PEI 4 and Alginate hydrogel	In this work, the applications of 3D-printing in Cardiac implants were included.	Izadifar, Chapman, Babyn, Chen, and Kelly (2018)
	Human coronary artery endothelial cells	CNTs 5, collagen (methacrylated) and Alginate hydrogel	Nanoreinforced cardiac patches (3D) were printed and assessed in the study.	Izadifar et al. (2018)

Vascular tissue	HUVEC 1	Gelatin ink (completed with PEG-SVA)	Hydrogels (3D) were printed, as in the study which proved to be compatible to the cells.	Rutz, Hyland, Jakus, Burghardt, and Shah (2015)
	Not specified	Sacrificial material made of networks of carbohydrate glass filament	The study dealt with the 3D-printing of networks of microvascular tissues.	Cui and Boland. (2009); Miller et al. (2012)
Nerve tissue	Frontal cortical human neural stem cells	Calcium chloride along with Alginate-CMC- agarose hydrogel	The study focused on the production of neural mini-tissues and it thus laid down several applications associated with human neural tissues.	Gu et al. (2018)
	RSC96 cell line	Calcium chloride/PEI and Alginate hydrogel	The study reported for the recovery of peripheral nerve injury by cellular reparation.	Rajaram Schreyer, and Chen (2012)

WAP: 1 human white adipose progenitor cells; *BAP*: 2 human brown adipose progenitor cells; *HWA*: 3 human adipose progenitor cells; *HUVEC*: 4 human umbilical vein endothelial cells; *PEG-4A*: 5 4arm poly(ethylene glycol) acrylate; *1 DMEM*: Dulbecco's modified eagle medium; *HDF*: 1 human dermal fibroblast; *2HEM*: human melanocytes; *HaCat*: 8 human keratinocytes; *NIH/3T3*: murine fibroblast cell line.

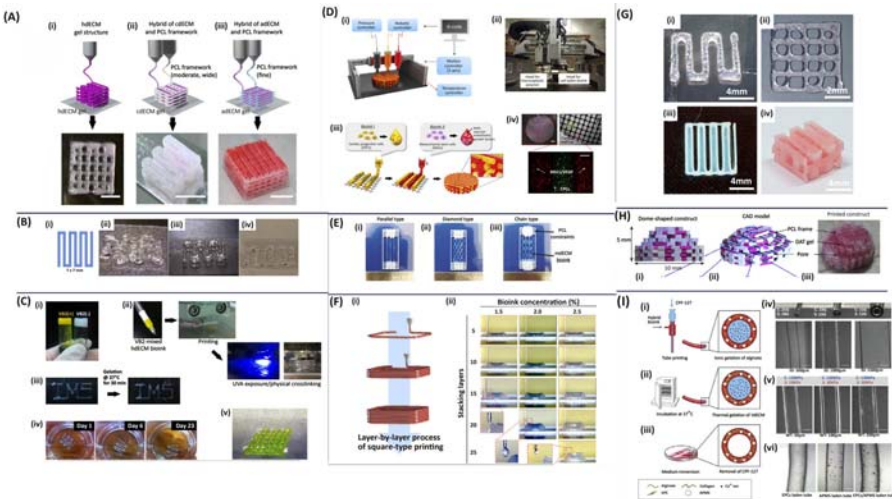


Figure 3.4 Decellularized extracellular matrix used as bioinks for the 3D printing of different tissue constructs using bioprinting techniques; **A** printing of Heart dECM cartilage and adipose tissue. **B** Printing of a liver dECM with Printing pattern design using polyethylene glycol (PEG) diacrylate and four-arm PEG alkyne-containing bioink and eight-arm PEG alkyne crosslinkers. **C** hDECm crosslinked with vitamin B2 in the presence of UVA light. **D** Printing of prevascularized stem cell patch printing system illustrating the fabrication of stem cell patch. **E** Cell-printed skeletal muscle constructs with CaCl_2 myoblasts and skeletal muscle dECM. **F** Cell-laden constructs of skin dECM bioink using stacking process illustrating layer-by-layer process of printing of various layers of bioinks with different concentrations. **G** 2D printing patterns of 3D hybrid structures with the polymer printed along with liver dECM bioink. **H** Decellularized adipose tissue constructs printed construct shows the PCL framework of DAT gel and pores. **I** fabrication a bio-blood-vessel using combination of hybrid dECM bioink with a coaxial cell printing system describes the step of process that is how the alginate undergoes ionic gelation with the crosslinking of collagen fibers resulting in the hybrid bioink.

Source: Reproduced with permission Choudhury, D., Tun, H. W., Wang, T., & Naing, M. W. (2018). Organ-derived decellularized extracellular matrix: a game changer for bioink manufacturing?. *Trends in Biotechnology*, 36(8), 787–805.

Three-dimensional bioprinting of organs

Organs are the top form of cell development. The simplest unicellular cellular entity (i.e., cell) is converted into complex and systematically arranged multicellular tissues that can perform a specific function. During an organ transplant, generally, there is always a risk of whether the body of a patient can accept the implant (organ) or not. The body recognizes it as a foreign body and starts reacting accordingly, resulting in a low rate of success. Moreover, the time spent on the search of a suitable donor sometimes creates a loss for the patient even when one finds a suitable donor. The cost which the patient has to bear for the purchasing of the

organ from the donor is another problem for a patient or family member. Time, cost, and low success rate are some of the basic drawbacks which compel researchers to think in a new direction. The success in the development of tissues under artificial environments led to the path for organ development. Organ printing is a process in which an organ of the living body is constructed artificially in a laboratory through a machine using appropriate environment and live cells having the ability to grow under experimental conditions. Although this is still at the experimental stage, it gives new light on the area of organ transplantation. Although scientists achieved a certain degree of success in the development of artificial hearts, kidneys, livers, cornea, etc., the research on organ printing is still in the process of development (Fig. 3.5). As bioprinted tissue is developed using the patients' cells, there is a lower risk of rejection after organ transplant compared to the traditional organ transplant, in which the donor's organ is treated as a foreign body by the patient's body. Nowadays, bioprinting is implemented for most of the body parts and the signs of progress have been demonstrated in Table 3.4, such as bionic ears, skins, artificial bones, vascular tissues, and cartilaginous structures.

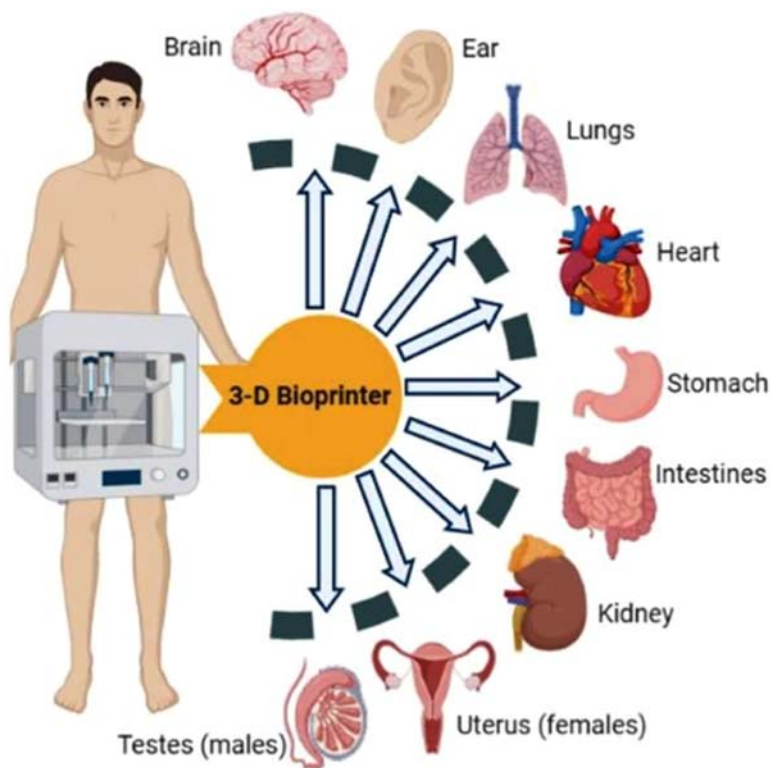


Figure 3.5 Applications of three-dimensional bioprinting in the development of different organs.

Table 3.4 Three-dimensional bioprinting used to develop artificial organs with biocompatibility.

	Cell lines	Material	Applications	References
Heart	HUVECs and MSCs	Polyester urethane urea	Cardiac patches	Gaebel et al. (2011)
	Cardiomyocyte progenitor cell (hCMPC)-	Alginate hydrogel	Cardiac construct	Gaetani et al. (2012)
	VIC seeded scaffolds	PEG-DA hydrogels and alginate hydrogels	Heterogeneous aortic valve constructs	Hockaday et al. (2012)
	Human aortic VICs	HAMA/GelMA-based hybrid hydrogel	3D simplified heart valve construct with root and trileaflets	Duan et al. (2013)
Liver	Primary hepatocytes	—	Liver tissue	Kizawa et al. (2017)
	hiPSCs: RCi-22 and RCi-50; and hESCs: RC-6 and RC-10	AlginateHydrogel (1.5% w/v)	Pluripotent stemcell	Faulkner-Jones et al., (2015)
	Hepatocellular carcinoma cell (HepG2) of human liver	Alginate hydrogel	Organized 3D architecture Lhepatocellular carcinoma cell	Chang et al. (2010)
	hiPSCs and hESCs	Alginate hydrogels,	3D-printed liver model	Ziogas et al. (2020).

Kidney	PTECs (2×10^7 cells/mL)	Gelatin/fibrin hydrogel (7.5% w/v, and 10 mg/mL respectively),	Human renal proximal tubules (PTs)	Homan et al., (2016)
	primary SMCs (isolated from rat bladder tissue) ($0.1-1 \times 10^6$ cells/mL)	Suspended in a collagen hydrogel (0.2% w/v).	Bladder tissue construct	Xu et al. (2010)
	with PCL/PLCL blend for fabrication of scaffolds, and extrusion of UCs (isolated from New Zealand white rabbits), and bladder SMCs (isolated from rabbit bladder)	Suspended in a gelatin/fibrin/hyaluronic acid hydrogel (35, 30, and 3 mg/mL, respectively).	Bioprinted urethra	Zhang et al. (2017)
	Primary SMCs (isolated from rat bladder tissue) ($0.1-1 \times 10^6$ cells/mL)	Suspended in a collagen hydrogel (0.2% w/v).	Bladder tissue construct	Xu et al. (2010)
Urinary bladder	Primary SMCs of rat bladder tissue	Collagen (0.2% w/v)	Bladder tissue construct	Horváth et al. (2015)
Brain	Primary cortical neurons.	Novel peptide-modified biopolymer, gellan gum-RGD (RGD-GG), hydrogel	Brain-like structure	Lozano et al. (2015)
	Zebra finch brain matrix	Agarose	Agarose brain mold	Huang et al. (2014)

GelMA/HAMA: Gelatin methacryloyl/hyaluronic acid methacrylate; *RGD*: arginine-glycine-aspartic acid; *hiPSCs*: human-induced pluripotent stem cells; *hESCs*: human embryonic stems cells; *PTECs*: proximal tubule epithelial cells; *SMCs*: smooth muscle cells; *hCMPC*: human cardiomyocyte progenitor cells; *VIC*: valve interstitial cells PCL/PLCL; *HUVECs*: human umbilical vein endothelial cells; *UCs*: urothelial cells.

The bioprinting of a live organ is a stepwise development of printing of living cells to tissue and from tissues to organ; therefore, with increase in the complexity of structure, the requirements also get specific. All the requirements can be categorized into three basic elements, such as biomimetic structure, biocompatible components, and biological microenvironment, where mechanical and chemical activities of the organ may be performed. Biocompatible components are well discussed under the section of bioink above. The knowledge about the bioink and the technique suitable for the bioprinting of the desired organ in its natural 3D structure facilitate the printing process. With the advancement in research, the alternative techniques that can be used and the bioink materials also increase, which are already discussed earlier in detail. The most crucial step in organ printing is associated with the postbioprinting process, which is responsible for providing a suitable biological microenvironment to the printed organ. These processes may be chemical, mechanical, or biological signals, which are essential in regulating the remodeling of tissue and its growth.

The development of new bioreactor technologies enables rapid maturation of tissues, multiscale vascularization for the survivability of tissues, and mechanical integrity and innervation for implantation (Choudhury, Tun, Wang, & Naing, 2018). In complex tissue/organ bioprinting, the bioprinting process still involves some major challenges like biomimetic and biodegradable printable materials, vascularization at cell level, maintaining cell viability until regeneration is completed (Matai et al., 2020).

Heart

The heart is the central muscular organ of the circulatory system of mammals, pumping blood to the whole body using the blood circulation system of the body. It is the first functional organ that evolved from the mesenchymal layer (splanchnopleuric) during embryonic development and is made of heart walls covering the four chambers and the four valves to maintain the unidirectional blood flow (Cui & Boland, 2009).

The complexity of the organ can be understood by understanding its physiological structure. The heart wall is constructed of three-layers; the innermost layer is the endocardium, which is made of endothelial cells, and protects the inner structures like valves and chambers; the middle layer is the myocardium (cardiomyocytes), which is a thick muscular layer that facilitates the contraction and relaxation process and the outer layer is epicardium or pericardium, which is a double-layered fibroserous sac, which helps in protecting the heart and controlling the overfilling of heart with blood (Cui & Boland, 2009).

It is clear from the structure of the heart that cardiomyocytes, fibroblasts, and endothelial cells are the three basic cellular components essential to printing this organ in *in vitro* condition. 3D bioprinting is a promising approach to reducing the challenges faced while regenerating the organ in *in vitro* conditions. According to the need of the patient, this technique is able to provide the required part for the proper treatment. Several types of research have been performed to prove the

efficiency of this technique. In this direction, [Gaebel et al. \(2011\)](#) used the laser-induced forward transfer (LIFT) cell printing technique used to develop the cardiac patches of polyester urethane urea (PEUU) seeded with human umbilical vein endothelial cells (HUVECs) and MSCs in a well-defined pattern to regenerate the cardiac patches ([Cui & Boland, 2009](#)). They also compared the 3D-printed patterned patch with the traditional random patches and found that the patterned patches show the enhanced vessel formation and improved functional properties. [Gaetani et al. \(2012\)](#) used the alginate hydrogel laded with cardiomyocyte progenitor cell (hCMPC) derived from human cardiac for the 3D bioprinting of the cardiac construct. They also examine the viability of the construct and found that the printed construct not only retained the phenotypic resemblance to the natural organ but also contain high cell viability ([Gaetani et al., 2012](#)). A detailed list of the attempt taken by the researchers has been summarized in [Table 3.4](#). Hockaday and his team used the dual-nozzle printer and tried to print the 12–22 mm inner diameter aortic valve with geometries ([Hockaday et al., 2012](#)). In a research by Duan and coworkers, encapsulating the smooth muscle cells (SMCs) of aortic root sinus in the valve root and aortic valve interstitial cells (VICs) in the leaflet were found viable over the 7 days in culture conditions. These encapsulated SMCs and VICs were found to express higher alpha-smooth muscle actin (α -SMA) and vimentin in the printed stiff and soft matrix, respectively ([Duan, Hockaday, Kang, & Butcher, 2013](#)).

Liver

The liver is responsible for controlling metabolism in humans. It regulates various metabolic activities like storage of excess glucose in the form of glycogen, decomposition of RBC, synthesis of plasma protein, production of various hormones, and detoxification process ([Cui & Boland, 2009](#)). Just like the heart, the liver is also divided into four lobes made of hepatic lobules. On microscopic examination, these lobules are hexagonal and made of hepatocyte plates emerging from the central vein. Liver sinusoids are a network of capillaries present between these plates and link the central vein with the portal triads. These sinusoids serve as an area for the mixing of oxygen-rich blood coming from the hepatic artery and nutrient-rich blood from the portal vein ([Cui & Boland, 2009](#)).

Histologically, parenchymal and nonparenchymal cells are the chief cell types responsible for liver development. Here, the nonparenchymal cells themselves are of various types, such as sinusoidal endothelial cells (SECs), phagocytic Kupffer cells (KCs), and hepatic stellate cells (HSCs), etc. The hepatocytes show high proliferation, which gives the liver its regeneration capacity. This unique characteristic of hepatocytic cells draws the interest of tissue engineers. Traditionally developed liver tissues have some basic limitations that 3D printing can resolve ([Ikegami & Maehara, 2013](#)).

In the initial works, Wang et al. developed a 38-layered assembly of hepatocyte/gelatin construct using the approach of 3D printing. The developed hepatocyte was also examined for its viability and found to remain viable for about 2 months and also able to perform the biological functions ([Wang et al., 2006](#)). In another work,

Li et al. used the double nozzle technique of 3D printing to construct the liver structure with a vascular network (Li et al., 2009). Chang et al. used the multinozzle-based extrusion system and hepatocellular carcinoma cell (HepG2)-laden alginate hydrogel to fabricate 3D architecture of the human liver (Chang, Emami, Wu, & Sun, 2010). These constructs are also successfully used in various assessments like drug toxicity, mode of action of various drugs, etc. Bhise et al. used these constructs in the form of liver-on-chip for the evaluation of drug toxicity (Bhise et al., 2016). A detailed list of the attempt made by the researchers has been summarized in Table 3.4.

3D printing technique showed its potential toward the construction of liver architecture with biological functions. However, to reach this, there are still some major hurdles like the availability of primary hepatic cells because it might be difficult to perform liver biopsies for each patient. Furthermore, several reports mentioned that primary hepatocytes lose their functional ability gradually under ex vivo conditions (Nguyen et al., 2016; Vijayavenkataraman, Yan, Lu, Wang, & Fuh, 2018). These reports compel researchers to also seek some alternatives to overcome this limitation. Pluripotent stem cells are used as an alternative to print hepatocytes or HLCs (Faulkner-Jones et al., 2015; Vijayavenkataraman et al., 2018; Crook & Tomaskovic-Crook, 2020). For the proper utilization of this technique in favor of mankind, some studies regarding protocol optimization, long-term postprinting functionality, and molecular pathways of the differentiation process are still required.

Kidney

The kidney is a bean-shaped structure that is present slightly posterior to the liver and below the liver. It is the central part of the excretory system of our body, which helps in removing waste or toxic material from the body and helps in maintaining the overall fluid balance of the body, filtering the minerals from the blood before sending them back to the heart. It also plays an important role in maintaining blood pressure, bone mineralization, and vitamin D synthesis (Scott & Quaggin, 2015). To print the live kidney in in vitro condition, it is important to know its structure at the cellular level. Externally, it is made up of three layers; the outermost layer is the renal fascia, which is a layer of tough connective tissue; the middle layer of perirenal fat capsules, which helps in anchoring the kidney in a definite position; and the innermost layer is the renal capsules, which is the shock-absorbing layer of adipose tissues. Internally, the kidney is also divided into three regions: the outer region cortex, the middle is medulla, and the inner region is the renal pelvis known as helum. As filtering of blood is one of the main functions of the kidney; therefore, the network of the blood vessel is an important component of the structure. The kidney is made of millions of tiny filters known as nephrons present in the cortex region and is the functional unit of the kidney. The nephron is divided into glomerulus and Bowman's capsule.

Current methods applied for the treatment of diseases related to these organs like reconstructive procedures with native neurologic tissues (skin or gastrointestinal

segments) are associated with complications that arise due to the incompatibility of the grafted tissues used to treatment the effects of long-term urine exposure (Korossis et al., 2006). Hence, artificial bioengineered bladders are needed. However, multiple functions and the complexity of an architecture is the main hurdle in the bioprinting of the biologically active kidney. Work in the area of bioprinting has also started quite late in the 1980s, wherein proximal renal tubular cells were seeded on the porous polymeric scaffolds. During this study, the human body environment was maintained while keeping the scaffold in the hemofiltration circuit (Madariaga & Ott, 2014). The construct was found to be able to perform the function of urine filtration, metabolism, cardiovascular stability; however, the life of the construct was still the major issue. The introduction of 3D bioprinting gives a new horizon to this field and till date few studies have been reported on the concept of bioprinting of kidney. Homan et al. (2016) attempted to print an open lumen 3D architecture of human renal proximal tubules (PTs), with enhanced epithelial morphology and functions as compared to 2D construct. Xu et al. (2010) used micro-valve bioprinting technique to print a tissue construct of urinary bladder using primary SMCs (isolated from rat bladder tissue) and found the postprinting viability of nearly N90%; however, the printed tissue was not able to maintain the morphological similarity from the native rat bladder in the histological studies. In another experiment, Zhang et al. (2017) used the PCL/PLCL blend for the fabrication of bioprinted urethra scaffolds, and found 80% postprinting cell viability was over 7 days of the study period with the morphological and mechanical similarity to the native tissue.

However, there are still many milestones that would have to be covered to get the functional bioprinted kidney. The architectural complexity of the organ demands an array of primary cells, which at present is unavailable. Similarly, the development of nephrons, which are approximately millions in a kidney, is itself a challenge. Bioink itself is in the very initial stages; studies are required for the formulation of an ideal bioink that will resemble the complex renal structure and functionality (Reint, Rak-Raszewska, & Vainio, 2017).

Brain

The brain controls voluntary and involuntary human activities. The brain is the only organ in which there are least chances of repairing in case of any damage. Furthermore, the intrinsic complexity of the organ creates challenges for neuropathology. A basic understanding of the cell types and their organization is essential to finding out the cure for any disease. Although a high degree of functional complexity is present in the brain, yet neurons and glia are predominantly present in the normal cells of the brain. Moreover, these two, endothelial, perivascular cells, leptomeningeal meningotheial and mesenchymal cells are also found. The morphology, organization, and functions of these cells vary considerably. Stem cells-assisted bioprinting is a combination of technologies showing tremendous potential in the revolution of neurosciences (Walus, Beyer, & Willerth, 2020). 3D tissue models are still in a very primitive stage. It has to cover several milestones, such as

the bioink formulation according to the complex multidimensional requirement, cell viability, and cell differentiation. Moreover, the developed 3D model should mimic the complex architecture and functions of the brain.

Conclusion

The technique of 3D bioprinting, which was primarily invented and intended to be used in the food sector for its applications in developing attractive and designer food products; has now been considered as a vital breakthrough in the medical and pharmaceutical sciences. The capability of the technique in the aforementioned domains can be traced by the manufacturing of the first ever 3D bioprinted tablet, which is under the lenses of FDA, awaiting approval. However, there exist several limitations in its medical applications related to the bioprinting status of the cell(s), tissue(s), or organ(s) printing, by knowing its promising future in various fields. Currently, many groups continue to find solutions to the existing problems in this area. Some of these worth mentioning innovations are the development of various bioinks with distinct characteristics; different bioprinting techniques that are responsible for the printing of different types of cells, providing contrasting physical properties; and the application of bioreactor technologies, which could add pace and maturity in the developmental process of 3D-bioprinted tissues or organs. With advancement in supporting fields like bioengineering, chemistry, computer science, mechanical engineering, robotics, cell biology, and material sciences, 3D bioprinting will soon reach its clinical translation in the near future.

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Three-dimensional printing of grafts and implants

4

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Introduction

Individuals aged 65 and above comprise nearly 10% of the world population today and are expected to double by the end of 2050. Old age is associated with health complications, such as bone fractures, tissue loss, and other age-related issues. Addressing these issues requires either fixation, reconstruction, or replacement of either the complete or part of the tissue. There has been an increasing demand in organ replacement or for tissue regeneration. Three-dimensional (3D) printing is providing a solution to the complexity of human tissues and bones and has proved to be better than other traditional or conventional techniques used. It is now possible to construct tissues with controlled porosity, mechanical strength, and permeability (Gopinathan & Noh, 2018; Murphy & Atala, 2014). 3D printing or additive manufacturing not only mimics the outer structure of a tissue or organ but also helps in creating the inner architecture of the tissue. The complete structure is the result of complex combination of specific biomaterial, cell source, vascularization, innervation, and maturation or its compatibility with the surrounding tissues. Although the field is new, it has already succeeded in bringing functionality in a tissue required for transplantation (Yan et al., 2018).

There has been a lot of research on printing of different types of cells with the functionality of human tissues or organs, especially for the soft tissues. The mechanical demands of hard tissues not only require the support and strength but also should be biocompatible enough to allow the growth of cells onto it without

any further complications. Currently, 70%–80% of the metals used in surgery include stainless steel, titanium alloys, cobalt-chromium alloys, nitinol, niobium, and tantalum. Recently, there has been a growing interest in other biodegradable metals, including zinc, iron, magnesium, and calcium (Ni et al., 2019; Xu, Wang, Sandler, Willfor, & Xu, 2018). 3D printing of different cells and tissues has been done using different techniques of bioprinting. Some of the specific tissues are discussed in the current chapter. The current chapter mainly focuses on grafts that involve different kinds of cells, metal printing for biomedical applications, and the use of microfluidics as new approach in 3D printing that can be used for different applications.

Three-dimensional printing of grafts or tissues

3D printing has been used to construct tissue or patch that can mimic the functionality of an organ. Different types of tissues fabricated using 3D printing are discussed next.

Cardiac tissue

Cardiovascular diseases are the number one cause of deaths globally and till date heart transplantation is the only solution for patients suffering from end-stage heart failure. Since there are a limited number of heart donors, there is a need to search for alternate methods to regenerate cardiac tissues. 3D printing can be applied to cardiac tissue engineering by integrating cardiac cells and polymeric biomaterials, which can act as sacrificial scaffold. These scaffolds provide the mechanical strength and promote the functionality of the tissue. After maturation, the printed tissue can be transplanted into the host where it can integrate with the surrounding cells and promote vasculature within it (Jakab et al., 2010; Li, Wu, Chu, & Gelinsky, 2020; Nabel, 2003).

Noor et al. performed the biopsy of an omental tissue of patient, which was reprogrammed to become pluripotent stem cells that were then differentiated to become cardiomyocytes and endothelial cells. The extracellular matrix was processed to form a hydrogel, which was then mixed with the two types of cells to form bioink (Noor et al., 2019). Similarly, Jang et al. were able to print human c-kit + cardiac progenitor cells using decellularized extracellular matrix. They used pneumatic pressure-based dispensing system connected with a heating unit, which was then connected to robotic microextrusion dispensing system. The printed structure had strong vascularization with tissue matrix formation. The printed structure had reduced cardiac hypertrophy and fibrosis with increased migration from patch to the infarct area (Jang et al., 2017).

3D printing is also being used by healthcare professionals to create patient-specific anatomic models. These models are not only for the instructional and

teaching purposes but also for advanced surgical procedural planning and decision-making in device choice and size. Instructional or information-based models include congenital heart defects, valve stenosis, and catheter-based valve implantation or repair procedures. In terms of surface spatial orientation and intracardiac anatomy, 3D-printed models scored higher than conventional imaging (Garekar et al., 2016; Vukicevic, Mosadegh, Min, & Little, 2017).

Scaffolds that promote viability and differentiation of cardiac cells have also been fabricated using 3D printing. Ho et al. fabricated polycaprolactone (PCL)-carbon nanotube composite and scaffold structure was obtained by 3D printing. The scaffold seeded with H9c2 cells was able to proliferate indicating the biocompatibility of the scaffold. The biodegradation of the scaffold was dependent on the Carbon Nanotubes (CNT) content (Ho et al., 2017). Similarly, Gaetani et al. 3D printed a patch comprising hyaluronic acid/gelatin matrix and human cardiac-derived progenitor cells as bioink with a surface of 2×2 cm and thickness of 400 μm . The bio-printed scaffold transplanted in a mouse model showed significant reduction in adverse remodeling and maintained the cardiac performance (Gaetani et al., 2015). Tijore et al. developed a 3D-bioprinted microchanneled gelatin hydrogel that not only supports the viability of cardiomyocytes but was also able to maintain the contractile functionality. The microchanneled hydrogel was found to be more aligned and demonstrated with synchronized beating as compared to the unpatterned hydrogel (Tijore et al., 2018).

Nervous tissue

Injuries in the peripheral nervous system from accident, trauma, tumor, or other illness may cause partial or complete paralysis in patients. The only current treatment is the suturing of distal and proximal nerve ends without introducing tension or placing an autologous nerve graft from a different location in the body. The former one is usually done for shorter nerve gaps (<5 mm) while the later is done for larger defects. Since the autograft procedure requires to harvest the graft using surgery, it may lead to loss of function and neuroma formation. Furthermore, there is also a chance of mismatch (diameter size or branched nerves or length), thereby limiting its application. Thus, there is need to develop certain alternatives to the autografts, which is personalized according to the patient's need (Bellamkonda, 2006; Gu, Ding, & Williams, 2014; Kim, Haftel, Kumar, & Bellamkonda, 2008).

Nerve guidance conduits is a potential alternative to repair large-gap nerve injuries where a tubular structure acts as a guide to the regenerating axons that can bridge the gap between the injured nerve (Lackington, Ryan, & O'Brien, 2017). Johnson et al. combined 3D imaging and 3D-printed nerve regeneration pathway incorporating both physical and biochemical cues. The printed structure had micro-grooves and growth factors to form bifurcating anatomical geometries. 3D imaging from the patient provided specific anatomies to print scaffold using microextrusion technique. The scaffold provided axonal guidance and chemokinetic functionality.

In vivo studies suggested that the 3D-printed scaffold can help in functional regeneration of a 10 mm nerve gap (Johnson et al., 2015).

Zhu et al. were able to design and print nerve guidance conduits of various shapes, sizes, and complexities. As demonstrated in Fig. 4.1, they were able to print human facial conduits. In vivo results indicate that implantation with microchannels were able to regenerate sciatic nerves extending toward the distal end of the injury site. Branching was observed across the entire length of the conduit with motor functionality and sensation (Zhu et al., 2018). Qian et al. combined 3D printing and layer-by-layer casting method for layer-by-layer porous scaffold fabrication. The 3D printer was composed of a rolling tube and a sprayer where the nozzle sprayed different solutions at constant speed. The innermost part consisted of arginylglycylaspartic acid (RGD) and polydopamine, which will be beneficial for cell adhesion and proliferation, followed by graphene/PCL dichloromethane and crosslinked with RGD and polydopamine layer. The solution was sprayed again to strengthen the tubular structure. In vivo studies suggested axonal regrowth and remyelination after peripheral nerve injury (Qian et al., 2018).

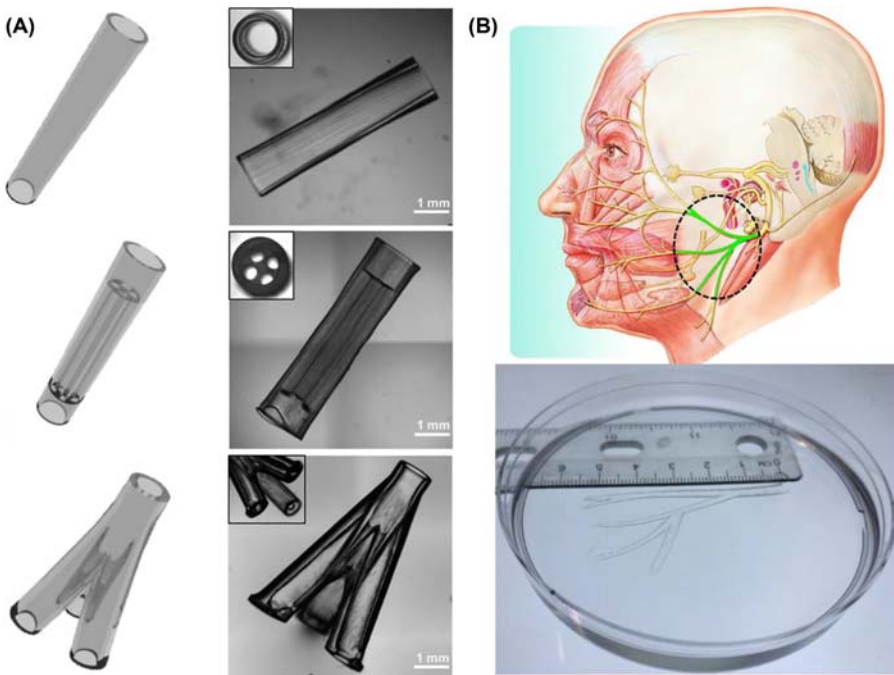


Figure 4.1 (A) Computer-Aided Design (CAD) designs and the corresponding 3D-printed structure of various geometry (B) facial nerve schematic and 3D printed structure. 3D, Three-dimensional.

Source: Reproduced with permission from Zhu, W., Tringale, K. R., Woller, S. A., You, S., Johnson, S., Shen, H., et al. (2018). Rapid continuous 3D printing of customizable peripheral nerve guidance conduits. *Materials Today*, 21(9), 951–959.

3D printing can overcome the limitations of conventional techniques by having control over the geometry and incorporating live cells and growth factors. It also helped in creating personalized conduits using imaging techniques and also with the functionality (Liu et al., 2022). Xu et al. incorporated RGFP966 encapsulated polymeric nanoparticles in the inner side of the nerve conduit. Digital light processing was used to print the conduit. RGFP966 activates PI3K–AKT–ERK signal pathway, which promotes remyelination of Schwann cells. In vivo results suggest biocompatibility and functionality of the conduit where it was able to regenerate a 10-mm rat sciatic nerve defect (Xu et al., 2019).

Skin

Skin plays an important role in the human body. It not only provides protection from the external environment but also helps in homeostasis, thermoregulation, and sensation. Patients suffering from acute wounds, burns, or chronic ulcers are affected with skin defects. The replacement of skin tissue should not only ensure the skin architecture but also the functionality. There has been a lot of research on skin replacement with some success as substitute; however, it has not been able to overcome for full thickness skin loss (Hierner et al., 2005; Tarassoli et al., 2018).

Koch et al. developed a multicellular 3D skin construct using laser-assisted technique. Fibroblasts and keratinocytes were embedded in a Matrigel scaffold (collagen and elastin) and deposited cells in a thin film. The cells in the printed scaffold were able to proliferate and differentiate. They also observed adhering and gap junctions, an important phenomenon for tissue morphogenesis and cohesion. The build-up of the basal lamina and Cx43 expression (gap junction) indicates skin tissue formation with the functionality of skin for direct exchange of metabolites, hormones, electric signals, and second messengers needed for physiological activities (Koch et al., 2012). Scaffold-free printing has been demonstrated by Pourchet et al., wherein they have fabricated bioink consisting of bovine gelatin, very low viscosity alginate, and fibrinogen. The bioink was mixed with human dermal fibroblasts and crosslinked in CaCl_2 solution. The printed structure was then seeded with normal primary human epidermal keratinocytes to generate a functional bioprinted skin. The authors were able to print an adult size ear of about 8 cm using bioink composed of human fibroblasts (Pourchet et al., 2017).

The bioprinted skin should also provide structural similarity to the natural extracellular matrix (ECM) and for its application in wounds and burns, the construct should have an adhesive property to fix at the site of injury. Zhou et al. developed a bioink composed of GelMA and *N*-(2-aminoethyl)-4-(4-(hydroxymethyl)-2-methoxy-5-nitrosophenoxy) butanamide (NB)-linked hyaluronic acid, which can be crosslinked using photo-initiator lithium phenyl-2,4,6-trimethylbenzoylphosphine and adhere at the site of injury. They used digital light processing to print a construct consisting of human skin fibroblasts and human umbilical vein endothelial cells. The printed construct had interconnected microchannels that helped in cell

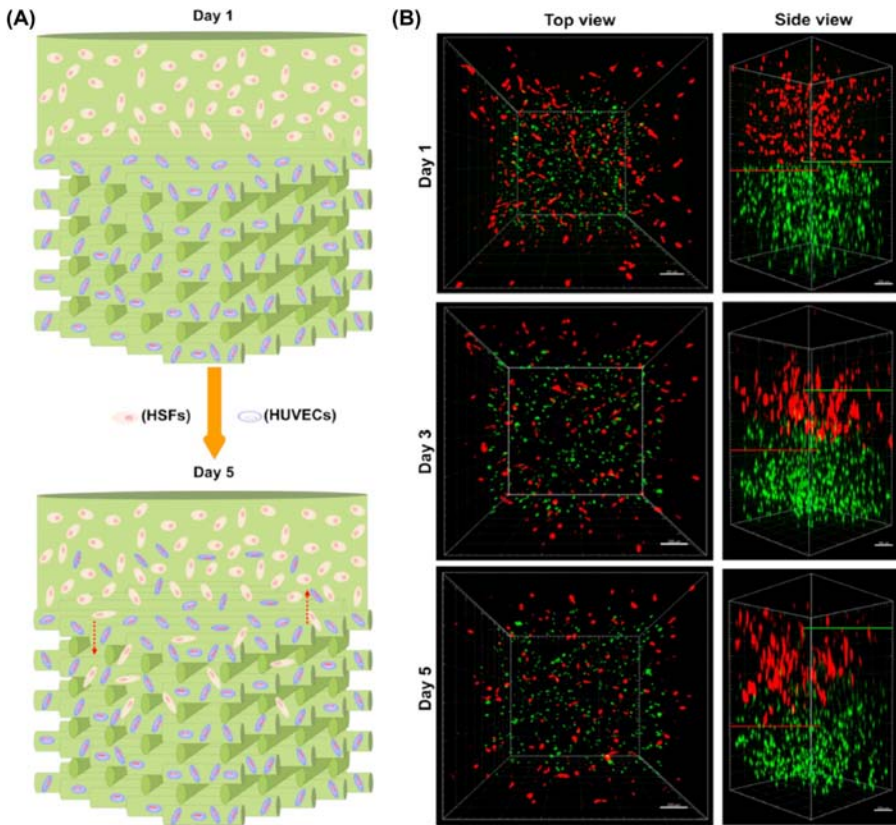


Figure 4.2 Cell migration analysis in the bioprinted skin construct: (A) schematic presentation of the cell migration, (B) human skin fibroblasts were labeled in red and human umbilical vein endothelial cells were labeled in green migrate after day 5.

Source: Reproduced with permission from Zhou, F., Hong, Y., Liang, R., Zhang, X., Liao, Y., Jiang, D., . . . Ouyang, H. (2020). Rapid printing of bio-inspired 3D tissue constructs for skin regeneration. *Biomaterials*, 258, 120287. <https://doi.org/10.1016/j.biomaterials.2020.120287>.

migration, proliferation, and neo-tissue formation. As shown in Fig. 4.2, human skin fibroblasts were labeled in red and human umbilical vein endothelial cells were labeled in green; the cells were able to migrate between the two layers. The interconnected microchannel structure not only helped in cell migration but also can promote oxygen or nutrient perfusion and neovascularization (Zhou et al., 2020).

Physical stabilization of the printed structure is also important in skin tissue engineering. Contraction in the printed structure fails to mimic the specific micro-environment and elasticity of the skin. Kim et al. decellularized porcine skin and used it as a bioink to print skin patches. The decellularized bioink had the

advantage of growth factors and cytokines required for the normal functioning of the skin. They were also able to encapsulate endothelial progenitor cells and adipose-derived stem cells in the printed structure and promote neovascularization (Kim et al., 2018). There is also a growing interest in 3D-printed wearable electronics and electronic skin wherein 3D-printed flexible and stretchable electrodes can be inserted into the skin for real-time monitoring of physiological signals in the body (Liu et al., 2018) or microneedles that can be used for drug delivery, such as in the case of insulin (Pere et al., 2018). The discussion of this research area is outside the scope of this chapter.

Liver

Liver not only metabolizes drug and food that we intake but also plays a major role in innate and adaptive immunity. Diseases such as acute liver failure, fibrosis, hepatitis, or chronic liver disease have increased in the recent years. Liver cancer is one of the five cancers with increase in annual occurrence (Anwanwan, Singh, Singh, Saikam, & Singh, 2020). This requires complex surgeries, such as hepatectomy and liver transplantation. In cases of end-stage liver diseases, liver transplantation is the only option. However, this strategy is limited due to a number of factors, such as surgical complication, immune rejection, or lack of liver donor. Regenerative medicine has proved to be an alternate solution to this problem. 3D printing not only helps in overcoming the complex anatomical structure but also to mimic the functionality of a liver (Wang et al., 2018). Applications of 3D printing for liver tissue engineering have been summarized in Fig. 4.3.

A major application of 3D printing in liver tissue engineering is to print a prototype model based on patient's Computerized Tomography (CT) and Magnetic

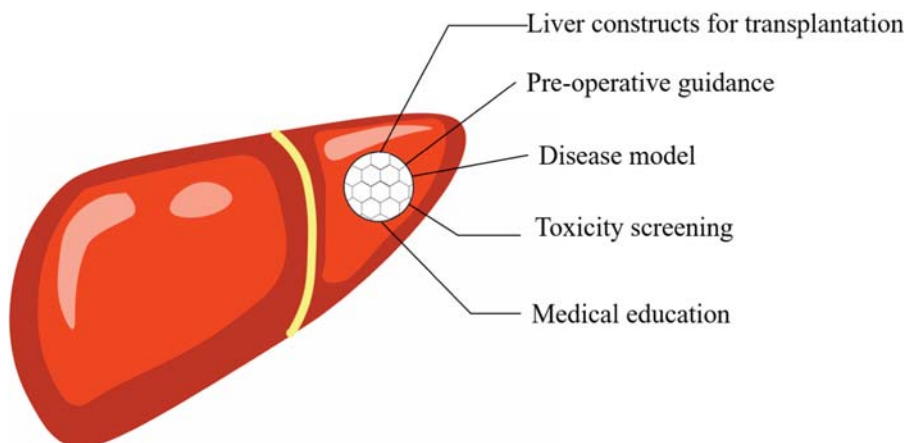


Figure 4.3 Application of three-dimensional printing for patient-specific liver models.

Resonance Imaging (MRI) images. The complex anatomical structures help facilitate surgery and minimize the intraoperative complications. Zein et al. demonstrated three surgical cases wherein they have 3D-printed anatomically identical livers of the donor as well as the patient. The printed structure had same geometry and volume, which helped identify complex vascular and biliary structures and avoid unexpected surgical complications (Zein et al., 2013). Apart from model printing for surgeries, 3D printing has also been explored for hydrogel printing for detoxification, since one of the important function of liver is detoxification. Gou et al. printed a liver inspired device, which was able to capture toxins for detoxification. The printed structure was inspired by liver lobule microstructure consisting of polydiacetylene nanoparticles in hydrogel matrix (Gou et al., 2014).

Several attempts have been made to 3D print liver patches. Faulkner-Jones et al. printed alginate hydrogel with human-induced pluripotent stem cells and human embryonic stem cells embedded into it. The cells were then induced into differentiate into hepatocyte-like cells. The printed structure had viable cells, similar morphology of hepatocytes and was able to secrete albumin and nuclear factor 4 alpha (Faulkner-Jones et al., 2015). Lee et al. developed a bioink from porcine liver by decellularization process. The decellularized bioink retained the ECM characteristics. Compared to collagen bioink, decellularized bioink was able to induce stem cell differentiation and retain HepG2 function (Lee et al., 2017).

Three-dimensional printing of metals as implants

3D printing of metals poses great advantage for biomedical application. Metals are preferred over biomaterials, such as polymers and ceramics for orthopedic applications, as they have better mechanical strength and excellent load-bearing capacity. Some of the main advantages are: (1) for the purpose of printing complex structures, which was difficult to achieve using only molding or casting methods. It comprises implants including hip, shoulder, knee, or for oral applications; (2) personalized implants: these address the issue of slight differences in the anatomy and structural geometry of different individuals. Personalized implants are helpful in overcoming the cases of poor adaptability, loosening, wear and tear, and meet the requirements of each individual patient; (3) porous implant metal: this is particularly true in cases of bone tissue implant where a stress shielding effect occurs due to the mismatch of mechanical strength and elastic modulus between the implant and the natural composition of the bone; (4) personalized surgical tools: offer more precision, speed, and efficiency when used in major surgeries; (5) medical devices: can be used for research and training purposes for medical practitioners to practise surgical procedures enabling more accurate and safer operations to patients (Bose, Vahabzadeh, & Bandyopadhyay, 2013; Ni et al., 2019; Vaz & Kumar, 2021; Yan et al., 2018). Metallic biomaterials should have strength that is comparable to the natural bone—not to create stress shielding effect but should have load-bearing

capacity, high corrosion resistance, high wear resistance, and low friction and biocompatibility. Some of the metals that are used as implants—titanium, iron, magnesium, and zinc, are discussed next.

Titanium

Titanium and its alloys have been used as implant material since 1970s for dental or as bone screws and plates or for artificial joints. They are biocompatible, good corrosion resistance, osteointegration, and high ratio strength. The titanium implants in more than 90% of the cases involve titanium alloy $\text{Ti}_6\text{Al}_4\text{V}$ (Ti-64) and commercially pure titanium (CP-Ti). However, they are also associated with the stress shielding property. To perform the natural functionality, it is necessary to match with the mechanical property of the bone (Popov et al., 2018). Compared to stainless steel and cobalt-chrome, titanium is a low modulus metal (110 GPa). However, when compared to the natural human bone (7–30 GPa) the modulus is about six times higher. This mismatch in Young's modulus creates a stress shielding effect—the bone surrounding the implant loses its load-bearing capacity, resulting in fractures at the implant site. Additionally, an unstable interface is created at the implant site of titanium and bone due to bone resorption during the bone remodeling process. 3D printing can be used to create a porous structure that can help lower the modulus significantly and also allow cells or tissues to grow within it (Dabrowski, Swieszkowski, Godlinski, & Kurzydowski, 2010).

3D printing for orthopedic and for dental implants has been done in various complexities to overcome the stress shielding effect. Xiao et al. printed mandibular prosthesis using titanium-coated polymer lattices to overcome this issue by functionally graded lattice prosthesis that can distribute the stress to overall structure instead of one focal point. This design can better adapt to the mechanical and biological functionality of the bone. The PLA polymer was 3D printed and a CP-Ti film was deposited with a thickness of 180 nm using radiofrequency magnetron sputtering at room temperature. The thickness of the film can be controlled by the sputtering time. Thus, titanium-coated lattices can withstand compressive strains exceeding 20% and the porosity of the printed mandible attained the maximum and minimum stress regions of 68.3% and 86.3%, respectively (Xiao et al., 2020). In another study, CP-Ti was 3D-printed using polyvinyl alcohol (PVA) as a binder. The printed structure had dual porosity features—micropores (residual pores obtained by binder burnout) and the macropores obtained by the design using a computer model. The printed structure had a porosity of 32.2%–53.4% and a compressive modulus of 0.86–2.48 GPa which is comparable to a cancellous human bone modulus (El-Hajje et al., 2014).

3D-printed implants are also being commercially used in surgeries. In one of the surgical case, a patient with degenerative cervical problems had a 3D-printed titanium implanted surgically. The printed structure was personalized trabecular bone prosthesis with nanostructure that helped in healing and fusion of the patient's bone. The titanium implant was printed using selective laser melting (SLM) that had the similar

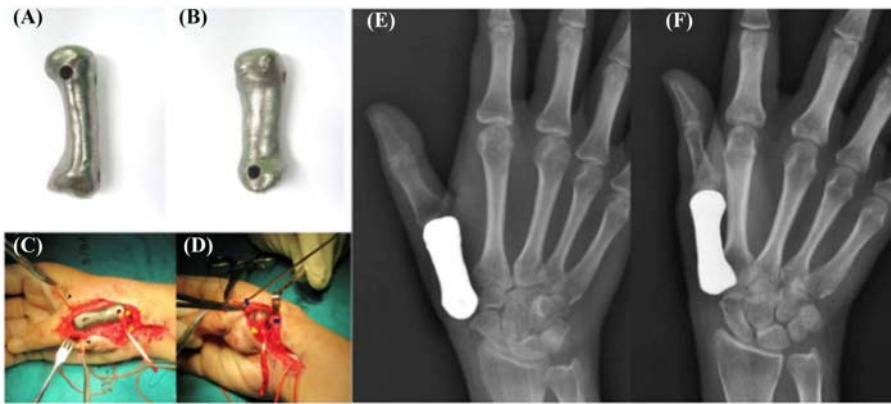


Figure 4.4 (A) and (B) Three-dimensional printed structures of anterior aspect and volar aspect of prosthesis before implantation; (C) and (D) intraoperative photographs with the 3D printed implant; (E) and (F) radiographs of the patient with the implant.

Source: Reprint with permission from Ni, J., Ling, H., Zhang, S., Wang, Z., Peng, Z., Benyshek, C., et al. (2019). Three-dimensional printing of metals for biomedical applications. *Materials Today Bio*, 3, 100024 and Punyaratabandhu, T., Lohwongwatana, B., Puncreobutr, C., Kosiyatrakul, A., Veerapan, P., & Luenam, S. (2017). A patient-matched entire first metacarpal prosthesis in treatment of giant cell tumor of bone. *Case Reports in Orthopedics*, 2017.

biomechanical property. The personalized and porous nature of the implant allowed better vasculature, better-fitting, better osteointegration, and improved patient compliance (Spetzger, Koenig, & others, 2017). Similarly, a 3D-printed titanium prosthesis was implanted in the palm of a female patient in the year 2015 as depicted in Fig. 4.4. The phalanges of the patient deteriorated due to cancer which was then replaced with a 3D printed titanium alloy with much flexibility. The successful implantation paved the way for other bone replacements (Punyaratabandhu et al., 2017).

Iron

Unlike titanium, iron comes under the category of biodegradable metals along with zinc and magnesium (which are discussed in the later sections) eliminate the risk of inflammation in the long run as they are absorbed in the body rather than be permanently in the body. The permanent bio-inert implants suffer from complications; such as chronic inflammation, thrombus formation, vascular abnormalities, infection, painful surgeries for removal of implants. Iron has better mechanical and biocompatibility as compared to magnesium and zinc-based implants. Additionally, iron has certain advantages that allow its use for orthopedic and dental applications. Iron is naturally found in abundance, easy to manufacture, biocompatible and biodegradable in nature. Iron is an essential element found in human body and is required for oxygen transport, electron transfers and also as a catalyst in some

enzymatic reactions. Iron is also essential for bone regeneration and in osteoblast development and differentiation. Iron also induces platelet activation, an important step during the healing stage of bone recovery. Similar to titanium, porous iron-based implants have been 3D-printed that allows to match the mechanical properties with the natural bone and also accelerate the biodegradation process and resorbed into the body which is a rather slow process (Balogh, Paragh, & Jeney, 2018; He et al., 2016; Hermawan, 2018; Putra et al., 2021).

Iron is one among the first materials 3D-printed for biomedical applications. Chou et al. 3D-printed Fe–30Mn scaffolds having a porosity of 36.3%. The printed alloy after sintering formed a mixed phase alloy of martensite ϵ and austenite γ phase. The biodegradation of the printed porous scaffold was higher than that of pure iron and the corrosion products contained calcium and phosphorus—both of which are biocompatible in nature. The printed structure was thus biocompatible and had similar mechanical properties as that of the bone overcoming the problem of stress shielding effect (Chou et al., 2013). Addition of Mn significantly increases the degradation rate, as much as twice as compared to pure iron. However, the corrosion products form a thick layer of calcium phosphate and oxide hindering the oxygen permeability and thus slowing down the degradation process (Kabir, Munir, Wen, & Li, 2021).

Though iron poses a number of advantages, the toxicity of the corrosion products still remains a concern. Iron scaffolds were fabricated using 3D printing and then debinding and sintering by Putra et al. The fabricated scaffolds had a porosity of 67% and pore interconnectivity of 96%. α -iron phase was present in the scaffold without any residual ink after the sintering process. The macro and micropores present in the scaffold improved the corrosion rate with 7% mass loss after 28 days; however, the mechanical properties were still in the range of that of human trabecular bone. However, the authors also observed a reduction in cell count, which was due to the high concentration of released iron ions. The *in vitro* assay indicated the cytocompatibility of the corrosion products but an *in vivo* study is required to study the biocompatibility of the implant and the toxicity of the corrosion products (Putra et al., 2021). A similar observation was seen by Li et al. where they were able to 3D print iron scaffold using direct metal printing with controlled topology and interconnected pores. After 28 days of immersion tests, the elastic modulus and yield strength of the printed scaffold decreased to 7% and 5%, respectively. The mechanical properties were in the range of trabecular bone even after the degradation. The porous structure helped the degradation rates to be 12 times higher than the commercial iron indicating that the structure and design played an important role in the degradation of the implant. However, cytotoxicity was observed when the implant was made to contact with MG-63 cells. In both the cases, an *in vivo* study has not been performed (Li et al., 2018).

Zinc

Zinc is also a biodegradable metal and is being explored as a scaffold for orthopedic and dental applications. Biodegradable metals meet all the requirements

considered for a bone implant—(1) mimicking the mechanical properties, (2) porous network for proper vasculature and growth of cells, (3) biodegradable without any toxic by-products. Among the common biodegradable metals, zinc is preferred over iron and magnesium, because its corrosion rate is close to the rate of tissue regeneration, whereas, for iron is slow and magnesium is faster (Bowen, Drelich, & Goldman, 2013; Su et al., 2019). Zinc is also an essential element in human body. Zinc is biocompatible and is required by the body in various forms especially in enzymes and in protein domains. It has a number of catalytic and regulatory functions in human body, for growth, development, and at cellular levels for differentiation, proliferation and apoptosis. It also acts as a cofactor in some enzymatic reactions involving vitamin A metabolism, DNA expression, membrane stabilization and useful in gustatory and olfactory systems (Agnew & Slesinger, 2020). However, the only disadvantage of using zinc is the low mechanical strength—low fatigue strength and creep resistance. 3D printing of zinc alloys is done to improve the mechanical property.

Cockerill et al. combined the process of casting and 3D printing to fabricate zinc bone scaffolds with pore size of 900 μm and 2 mm. The resulting scaffold had an interconnected pore structure. Increasing the pore size and porosity, decreased the mechanical strength but the corrosion rate was increased. The *in vitro* also confirmed the biocompatibility of these scaffolds (Cockerill et al., 2020). Li et al., 3D printed a scaffold comprising of PLGA/PCL/HA:Yb/Ho/Zn. The scaffold had better mechanical strength and slow degradation compared to PLGA/HA:Yb/Ho/Zn. It was observed that PLGA/PCL/HA:Yb/Ho/Zn scaffold was more conducive for bone reconstruction. The antibacterial activity of zinc exhibited promising potential for its use in defects with adequate mechanical support, degradation, antibacterial activity, and osteogenesis. The fluorescence activity of Yb/Ho ions and the high CT (X-ray) absorbency of Yb ions helped in *in vivo* tracking capability (Li, Zou, Wei, & Li, 2021).

Zinc plays an important role in growth and mineralization of bone. It activates aminoacyl-tRNA synthetase in osteoblastic cells and stimulates cellular protein synthesis (Guo et al., 2020). Wang et al., combined the advantages of PCL and zinc for fabricating bone scaffold. PCL is used for making porous scaffolds while zinc was used to increase the mechanical strength, osteogenesis and biodegradation. The composite scaffold was 3D printed using fused deposition modeling. *In vivo* results indicate that the scaffold promoted new bone formation after 8 weeks of implantation. The scaffold with 2 wt.% zinc displayed osteogenic effect by activation of the Wnt/ β -catenin and osteoclastogenesis by activation of NF- κ B signaling pathway (Wang et al., 2022).

Magnesium

Magnesium is also one of the biodegradable metals extensively studied for fabricating bone scaffolds. The advantage of using Mg-based scaffolds is that its modulus

is comparable to natural bone, thus minimizing the stress shielding effect (Chen, Xu, Smith, & Sankar, 2014). The disadvantage of using Mg-based scaffolds is their high degradation rate and high flammability of Mg powder. 3D printing is generally used for fabrication of porous materials, thus accelerating the rate of biodegradation. Mg can be used in combination with other polymers to overcome this disadvantage (Li et al., 2018).

Golafshan et al. were able to print an interconnected microporous structure using magnesium phosphate doped with strontium ions along with PCL. PCL provided the mechanical strength required to print complex geometry structure. The structure was printed using extrusion technique and had a compressive strength of 4.3 MPa. The scaffold induced bone formation without any osteo-inducing components in in vitro conditions and in vivo results suggest that over 6 months, the implant-induced bone regeneration without inflammatory reaction (Golafshan et al., 2020). Similarly Lai et al. printed bone scaffold by applying low-temperature rapid prototyping using Poly Lactic-co-Glycolic Acid (PLGA), β -tricalcium phosphate and Mg powder. The printed structure was not only able to mimic the geometry with mechanical properties but was also able to promote new blood vessel formation after 4 weeks of implantation. There was no increase in Mg ions in the serum after 12 weeks of implantation (Lai et al., 2019). Low-temperature rapid prototyping is a type of 3D printing where polymer solution is fused to form a scaffold in temperature less than -30°C . The polymer solidifies at low temperature and the solvent is removed by lyophilization.

Mg is essential for bone growth and metabolism. Its deficiency is associated with a variety of bone metabolic disorders. Mg_3PO_4 is generally used for fabricating scaffold since the dissolution products are Mg ions and phosphate ions, both of which are useful in bone repair. The concentration of Mg ions released from the scaffold is also important. A specific concentration is required for proliferation, osteoblastic differentiation, and migration while a high concentration can hinder new bone formation. Thus, controlled release of Mg ions from the degraded scaffold is also crucial (Shen et al. 2019, 2021). Lei et al. observed that 3D printed PCL scaffolds containing 20% $\text{Mg}_3(\text{PO}_4)_2$ were able to better promote osteogenic differentiation than PCL scaffold without magnesium (Lei, Gao, Zhang, Yi, & Zhou, 2022).

Conclusion

Research in 3D printing technology is rapidly increasing. Various scaffolds have been printed to achieve mechanical strength, ECM properties, immune acceptance, viability, and differentiation. The advancement in speed, precision and tunability allows improvement in printability of the scaffold. It addresses the major issue of shortage of donor organs for transplantations. Although there have been a number of successful attempts to mimic the anatomy and functionality of natural tissues or bone, there still exists certain issues that needs to be addressed before its

application at a large scale. The first major hinderance is the cost—both for manufacturing a 3D printer and the printed product. Second is the bioink—long-term studies are required to fully understand the application of these bioink at translational aspect. Natural polymers are biodegradable and biocompatible, but lack mechanical strength whereas metals used in implants have high elastic modulus resulting in a mismatch with the natural microenvironment. Third, apart from the decellularized tissue used as a bioink, it is difficult to mimic the structural components of ECM. Fourth, stacking of cells in a hydrogel matrix makes it difficult to supply nutrient and oxygen in the middle layers since the scaffold lacks the natural vascularization of the tissue. The imbalance in nutrient and oxygen supply creates difficulty in viability and differentiation of cells as compared to the cells present at the surface. Overcoming these limitations will help to fully explore the possibilities of 3D printing. Thus, 3D printing is still in initial stage but has a promising potential to be used in clinical applications.

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Three-dimensional printing for personalized medicine and targeted drug delivery

5

Chapter outline

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Introduction

Globally, millions of people are using some form of medicinal pills on a regular basis in the form of health supplements or in case of any illness. Every person is unique in terms of how they experience various physical, biological, or environmental factors. In addition,

there is a huge difference in food or living habits of people, if compared within the same or different geographical areas. These factors play an important role in developing the immunity of the person, which is the deciding factor of the human body to combat pathogens in the body or the extent of other immunity-based illnesses. All these factors are critical parameters to decide the treatment therapy in case of any illness. Due to the difference in the abovementioned factors, the actual dose required by a patient varies from person to person. However, in the current therapy, drugs are available in predefined concentrations at the pharmacy. Due to this, doctors are bound to prescribe these predefined concentrations, as a result, there are cases where the same concentration of the drug may be effective for someone or not effective for others. Overall, overuse or misuse of these medicines is very frequent, which ultimately plays an important role in the development of drug resistance: a serious concern in today's time. On the other hand, development of novel compounds and their clinical trials take nearly more than 10 years before they are available in the market. However, soon after their commercialization, resistant pathogens are also reported. This suggests microbes develop resistance rapidly than the discovery of new drugs. Therefore, there is an urgent need for development of more realistic biological models to accurately predict drug efficacy, with large pharmaceutical companies now subscribing to this "fail early and fail cheaply" mandate. These consequences show that the current medical practices also need reform in this sector. The concept of personalized medicine is thus emerging rapidly.

The emergence of personalized medicine: needs and advantages

Every person is different in terms of their immunity; it would be apt to say that every individual is affected by different combinations of diseases, hence the solution for every person cannot be the same. There are several genes responsible for the development of diseases, which can act as a molecular marker for specific diseases. Results of the Human Genome Project provide better insights into a human being and hence enhancing the adoption of genomics in medicine. Molecular pathways play an important role in the findings of novel potential targets. With advancements in genetics and molecular biology, researchers are able to develop a diagnostic kit using genes as molecular markers, which in turn will be useful for targeted therapy. Researchers suggest that the knowledge of variations in genes may lead to better and more realistic treatment with minimum side effects (Sandler & Preis, 2016). The concept of personalized medicine is the need of the hour and can change the picture of medical science. Physicians, by using diagnostic tests with specific biological markers, determine the most suitable drug combinations for the treatment of patients. Personalized medicine shows its potential in curing diseases related to genetics, environment, or medical histories, such as diabetes (Wei et al., 2009), cancer (Kim & shin, 2013), heart problems (Antman & loskalzo, 2016), and psychiatric diseases (Bzdok & Meyer-Lindenberg, 2018). One big challenge in the path of personalized medicine is to find out or predict drug response in an individual patient with accuracy (Zhang et al., 2018). However, improvements in

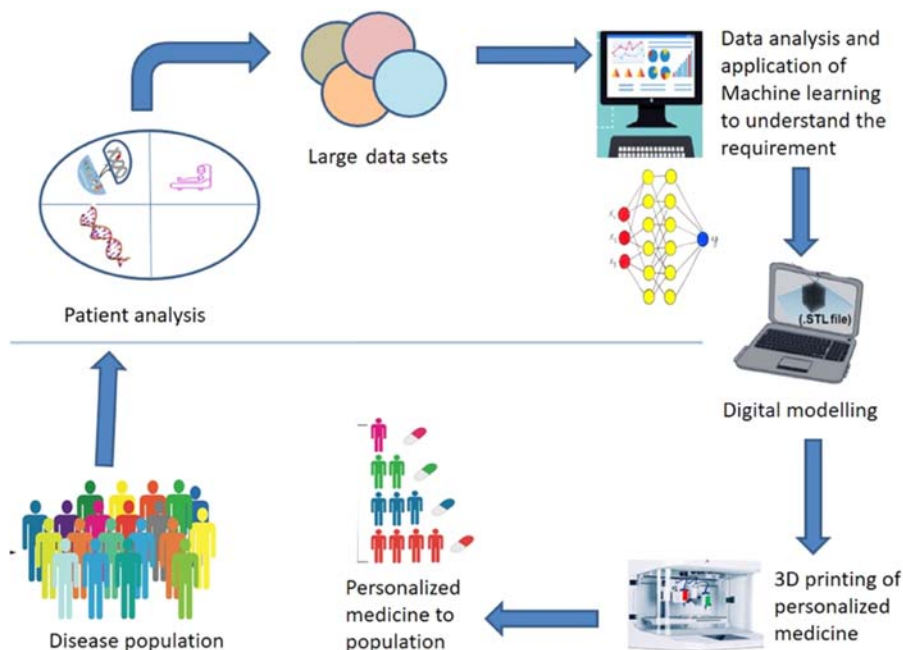


Figure 5.1. General process adopted for the development of personalized medicine according to the group of the patient.

machine learning programs of computer science play an important role in finding the solution to this problem. Zhang et al. in their article discussed in detail the perspective of deep learning in personalized medicine.

Fig. 5.1 depicts the general process of the development of personalized medicine for the patient. The machine learning approach is the application of artificial intelligence to develop new methods to analyze the data to find out any pattern for the prediction. Deep neural networks (DNNs) enabled the training of large data sets to identify important features. Ekins (2016) applies a deep learning approach in pharmaceutical research to predict the absorption, distribution, metabolism, excretion, and toxicity property of the lead molecules. Similarly, DNN is successfully applied in various areas of biomedicine field like drug discovery programs (Chen et al., 2018; Gawehn et al., 2016; Mamoshina et al., 2016), drug repositioning (Gittard et al., 2011), protein folding (Jo et al., 2015), etc.

Role of three-dimensional printing in personalized drug formulation

Formulation of any pharmaceutical product is an important aspect of the process as it is directly linked with the effect of the drug on the patient. Moreover, in case of

personalized medicine, it becomes more important because several criteria have to be kept in mind while designing a personalized medicine. It should include various characteristics of the disease, according to which the drug has to be designed, and also consider the effects of drugs. Some of them are depicted in Fig. 5.2.

Hydrophobic nature, that is, less solubility in the aqueous medium of the drug molecule is one of the major challenges in the drug formulation. Goke et al. (2018) proposed lipid-based colloidal drug delivery systems and the incorporation of the desired compound into nanospunfibers as novel strategies for the utilization of less hydrophilic compounds in the drug formulation.

Several 3D printing techniques are used in the manufacture of personalized medicines. Fused deposition modeling (FDM) is one of the most common techniques of 3D printing to develop personalized medicines. Kollamaram et al. (2018) used kollidon VA64 and 12 PF as potential materials for the printing of ramipril drugs through low-temperature FDM 3D printing and observed the drug degradation due to thermal heating during the printing process. They found that the ramipril did not undergo any degradation below its melting point.

Suitable dosage forms, including the consideration of individual release mechanism, rate, and profile, are highly required to prepare personalized medicine. Crushing or breaking tablets results in potent dosage form of drugs; however, this dosing is highly inappropriate and can be risky if used by an individual regularly. With the help of 3D printing technology, multiple personalized drugs can be formulated considering the individual release mechanism, rate, and profiles (Araújo et al.,

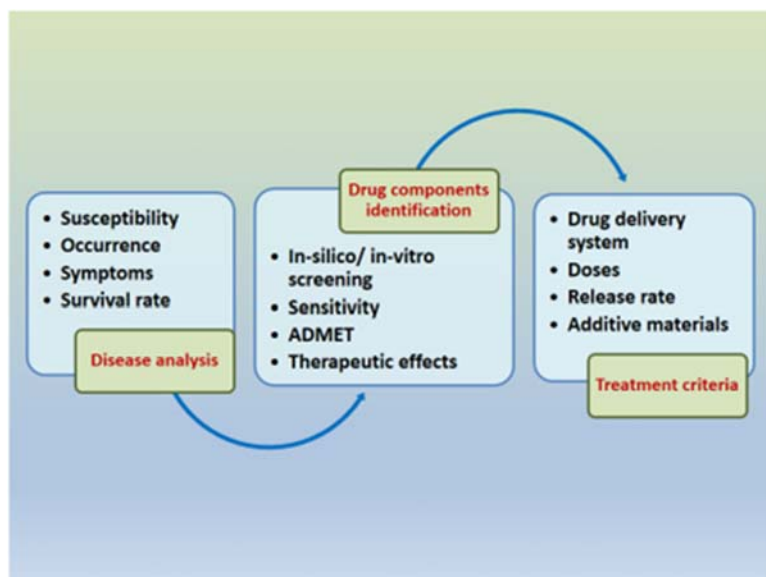


Figure 5.2. Important criteria for the development of personalized medicine for more efficient results.

2019; Narkevich et al., 2018). Compared to the traditional method, 3D printing for personalized drug formulation has higher effectiveness, accuracy, efficiency, and adaptability (Chen et al., 2020).

Application in oral dosage form

Oral forms are the most preferable and commonly used forms of medication. However, this form of medicine has considerable challenges related to people suffering from geriatric, pediatric, and dysphagia. Furthermore, rapid release of drugs is significantly helpful in diseases like hypertension (Pandey et al., 2020). Thus, these patients require a dose that they can take at night and achieve maximum concentration in the morning. For this kind of personalized dose, Zhao and coworkers developed a new model of a 3D-printed shell that can convexly release drug. In this model, they fill the mixture of PVA and drugs in a printed shell having internal tetrahedron cavity and successfully formulate a dose whose drug dissolution increases over time (Zhao et al., 2018). Yu and his team developed a tablet capable of disintegrating and wetting in 21.8 and 51.7 seconds, respectively (Yu et al., 2009). Another report related to accelerated drug release rate using FDM printer is presented by Yang and his team, which shows that the release rate of ibuprofen is highly affected by the patterns and size of tablets (Yang et al., 2018). Bilayer tablets of guaifenesin prepared using 3D printing technology have appropriate immediate release and sustained release layer than the marketed bilayer drug Mucinex (Khaled et al., 2014). A series of warfarin-containing tablets, produced by Tian and his team, has accurate dose (varies by the variation of tablet size), friability, and hardness (Tian et al., 2018).

The traditional method for the formation of polypills are costly, and the dosage is also not specific. 3D printing can easily be used to tailor the dosage with higher specificity considering the individual medication plan. Maroni and his team using FDM successfully developed the two-compartment capsular device to carry two incompatible drugs (Maroni et al., 2017). Christos and co-researchers applies this concept of compartmentalizing drugs to design a tablet with two antidiabetic drugs, namely glimepiride and metformin (Gioumouxouzis et al., 2018). In comparison to adults, pediatric populations have pharmacodynamics and pharmacokinetic properties, and thus they required significant care in optimizing the adverse effect of dose (Oblom et al., 2019). Adjustability in dosage is possible in sirups, but these are prone to error and have unpleasant taste (Scoutaris et al., 2018). An innovative solution to this issue is a mini-printer, which is produced by 3D printing. In one of the proposed studies, 3D-printed jolly-shaped chewable tablets of indomethacin were made, which masked the bitter taste (Scoutaris et al., 2018). Though multiple pieces of research show tremendous success in preparing personalized oral dosage drugs using 3D printing technology, these drugs may face massive rejection due to ethical issues and improper knowledge.

Application in transdermal dosage form

Transdermal delivery is highly effective in transporting the drug into systemic circulation via the stratum corneum of the epidermis (Sharma, 2018). Enormous

traditional techniques contributed to this kind of delivery by developing patches, iontophoresis, permeation enhancers, and sonophoresis (Economidou et al., 2018; Mehra et al., 2015; Prausnitz & Langer, 2008). Patches are designed in a pattern to consist multiple layers of drugs providing prolonged and continuous relief. Patches are developed either by compartmentalization or by creating a matrix. In compartmentalization, the drug is stored with the defined membrane; whereas, in matrix type, drugs are directly released into the skin (Jain et al., 2018). Asunder patches like Emsam (antidepressant drug), Exelon (for treating Alzheimer's symptoms), and Evra (contraceptives) are already available on the market (Abrams et al., 2002; Dhillon, 2011; Jessen et al., 2008). Traditional methods of microneedles preparation, such as microfabrication, involve multiple steps and are complex.

The application of 3D printing to fabricate microneedles and patches is suggested for enhanced specificity and activity. To combat this complexity, techniques like SLA and DLP are highly suitable for the manufacturing of microneedles (Chen et al., 2020). Boehm and his team used microstereolithography to design a microneedle for treatment and diagnosis of diseases (Boehm et al., 2014). Using a Perfactory III SXGA + printer, Gittard et al. developed a microneedle for wound healing taking acrylate-based polymer (Gittard et al., 2011). Furthermore, using the layer deposition method, an antimicrobial layer of zinc and silver oxide was coated on microneedles (Gittard et al., 2011). Lim and co-researchers developed a curved microneedle patch having varying curvatures using a DLP printer (Lim et al., 2020). Moreover, DLP printers are also used to print the microneedles masters upon which researchers cast polydimethylsiloxane to create its mold (Lopez-Ramirez et al., 2020). VAT printing is commonly used for 3D printing with photopolymerization having the advantage of high resolution, which is vital for designing microneedles with sharp tips with high skin penetration potential (Chen et al., 2020). However, a few disadvantages also reside in these techniques, like limited candidate materials for photocrosslinkable polymers and others.

Regulatory controls

The growing interest in using 3D printing technologies and advanced manufacturing in healthcare demands regulatory controls monitoring the production and application of printed products. FDA became the first worldwide regulatory system that provides a comprehensive and technical framework to advise and govern manufacturers responsible for creating products using 3D printing technology (Kelly, 2017). FDA launches initiatives for expanding the commitment to advance manufacturing. These initiatives are Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), and Center for Devices and Radiological Health (CDRH). CDRH, CDER, and CBER are focused on monitoring quality, emerging technology programs, and advanced technologies programs, respectively (Hahn, 2020).

3D-printed levetiracetam (Spritam) tablet, developed by Aprelia Pharmaceuticals, is the first drug that received approval from FDA to treat epilepsy (Reddy et al., 2020).

Investigational New Drug Application by FDA was received by Triastek for its first 3D-printed T19 drug (developed to treat rheumatoid arthritis) (Thakkar et al., 2021). CDRH approves multiple 3D-printed devices like bone plates, ear devices, surgical instruments, facial implants, and dental crowns (Hatton et al., 2018). However, for advanced manufacturing in the world of pharmaceuticals many regulatory and technical challenges must be considered. In addition, concerns about the safety of environment and humans are highly deemed to be accompanied by materials used, cost-effectiveness, and scalability. Moreover, the framework designed for 3D printing at the point of critical concern (US FDA., 2020) is summarized in Table 1. Nevertheless, the FDA has software programs specifically intended to generate 3D models of patients' anatomy. Still, it all depends upon the entire medical facility to use this software within the scope of its correct use.

Drug delivery

Route of drug administration is one of the important factors to achieving maximum drug response. Personalized drugs are patient-specific drugs although their introduction to the human body may be accomplished by various anatomic routes similar to the traditional drugs (Narkevich et al., 2018). Drugs are classified based on the route of administration or based on doses. The drugs can be administered either directly to the target tissue/organ or by systemic routes like gastrointestinal (oral, rectal), parenteral (subcutaneous, intramuscular, intravenous, intra-arterial) nasal, transdermal, pulmonary, and ocular. With the advent of 3D printing techniques more specific drug formulations with better results are being introduced.

Materials used in 3D printing to design personalized drug delivery

Drug formulations are generally either lipid-based or polymer-based. Furthermore, among the polymers, natural (proteins, collagen, gelatine, albumin), artificial (cellulose, ethylcellulose) and synthetic (biodegradable like polyester, polylactic acid; and nonbiodegradable acrylic, polymethyl methacrylate) polymers are used in the 3D printing of personalized drugs. All these polymers are discussed in detail in Chapters 2 and 3; however, some of the commonly used polymers are summarized in Table 5.2 and some are explained to understand the process of drug formulations.

Polyvinyl alcohol

Polyvinyl alcohol (PVA) having backbone formula $[\text{CH}_2\text{CH}]_n$ is a water-soluble, biocompatible, synthetic polymer that is prepared from the partial or full hydrolysis of polyvinyl acetate (Fig. 5.3). Due to its steric stabilization effect, PVA reduces protein adsorption on its surface; hence, it is used in the cell collection medium to study the biological functions of cells. Thermoplastic nature of PVA makes it suitable for use in additive manufacturing. The molecular weight of the polymer

Table 5.1. Critical concerned conceptual framework for three-dimensional printing.

S. No.	Context	Elucidation
1.	Healthcare facility becomes a manufacturer	A direct point of a care facility that wishes to print the devices and demand full authority over its applications and operations can become a manufacturer and thus be responsible for all regulatory framework accomplishment and requirements.
2.	Co-location of manufacture at the critical concerned point	This arises when a health point care facility and device manufacturer has the exact location.
3.	Turnkey system: Device designed by the manufacturer with the help of validated process	The manufacturer must receive a clearance report from FDA for its product application. For the approval, a demonstration of that specific product is required to evaluate its application needed by the end-user. Manufacturers must include the hardware, software, process parameters, and other required handling information in the finished devices. Healthcare is solely responsible for printing products and using the product within the approved specification obtained by the manufacturer.
4.	Additional potential requirements of professional healthcare: Device designed by the manufacturer with the help of validated process	The approved devices probably have a label that includes the supplemental instructions for the user. The approval process also involves the additional requirements for on-site training and testing from the manufacturer to facilitate the appropriate 3D printing.
5.	Nominal risk 3D printing	Devices that come under this context may pose a slight risk to patients. However, to meet an appropriate standard of the device, there is still a requirement of a defined Framework by the FDA, including the models used for patient counseling and education.

Table 5.2 List of some commonly used polymers in three-dimensional printing of medicine for targeted drug delivery.

S. No.	Polymer	Product	active pharmaceutical ingredient	Technique	References
1.	PVA	3D printed tablets	Carvedilol and haloperidol	FDM	Wei et al. (2020)
2.	PVA	Tablets with Multiple Release microneedle	paracetamol	FDM	Xu et al. (2019)
3.	PLA		estradiol valerate	FDM	Khosraviboroujeni et al. (2022)
4.	PLA	filaments and scaffolds	prednisolone or dexamethasone	FDM	Farto-Vaamonde et al. (2019)
5.	PVP	filaments	Dipyridamole or theophylline	HME	Okwuosa et al. (2016)
6.	PVP	Caplet shaped tablet	Dipyridamole or theophylline filaments	FDM	Okwuosa et al. (2016)
7.	PCL	tablets	fluorouracil	selective laser sintering	Salmoria, et al. (2017)
8.	PCL	biomimetic scaffold	chondrocytes	FDM	She et al. (2021)
9.	PEG	fast drug release tablets	pantoprazole sodium	FDM	Kempin et al. (2018)
	PEG	Hydrogels	resin	light-based stereolithographic	Benjamin et al. (2019)
10.	Alginate-methacrylate	Microneedles for Microencapsulated Cell Extrusion	human hepatocellular carcinoma (HepG2) cells	stereolithography	Farias et al. (2018)
11.	PCL-alginate	dual-drug-releasing scaffold to loaded alginate hydrogel	cefazolin (CFZ)-rifampicin (RFP)-	FDM	Abasalizadeh et al. (2020)
12.	PCL- chitosan	Implants	ibuprofen	FDM and HME	Yang et al. (2022)

FDM, Fused deposition modeling; PVP, polyvinylpyrrolidone; HME, hot-melt extrusion; PCL, polycaprolactone.

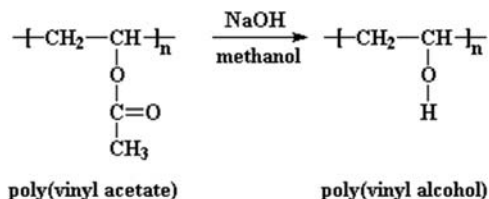


Figure 5.3. Scheme of formation of polyvinyl alcohol from polyvinylacetate.

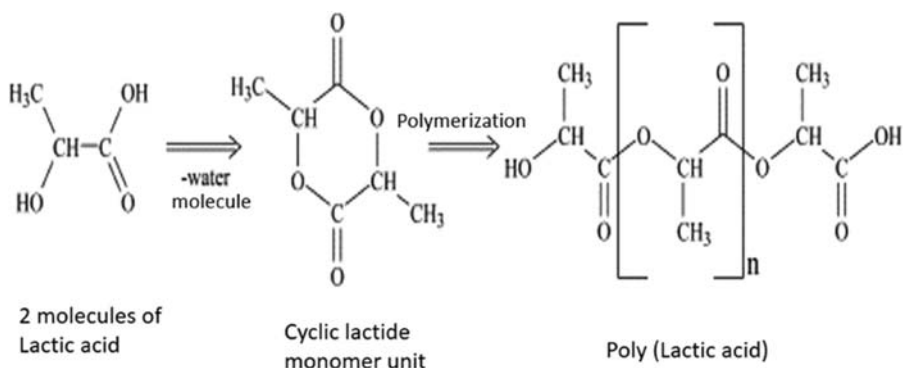


Figure 5.4. Scheme of formation of polylactic acid from lactic acid.

affects the viscosity of the prepared bioink, which in turn is responsible for the printability. The range of molecular weight of PVA makes it suitable as per the demand of viscosity range and 3D model.

Poly(lactic acid)

Poly(lactic acid) (PLA) having $[-C(CH_3)HC(=O)O-]_n$ backbone structure is generally formed by the condensation polymerization reaction of lactic acid (Fig. 5.4). It can also be prepared by the ring-opening process of lactide. Approval of PLA as a safe polymer by the US Food and Drug Administration (FDA) makes it the second most widely used biopolymer in the field of controlled drug delivery, tissue engineering, and regenerative medicine. Due to its nontoxic and noncarcinogenic nature, it is generally used for SLA and FDM printing for various human or animal studies at the cellular level (Stansbury & Idacavage, 2016).

Poly (caprolactone)

Polycaprolactone (PCL) is a biodegradable polyester having backbone $[-CO(CH_2)_5O-]_n$, which is generally synthesized either by ring-opening polymerization or the condensation cyclization polymerization reactions as mentioned in the scheme (Fig. 5.5). It is hydrophobic and semicrystalline in nature and its crystalline

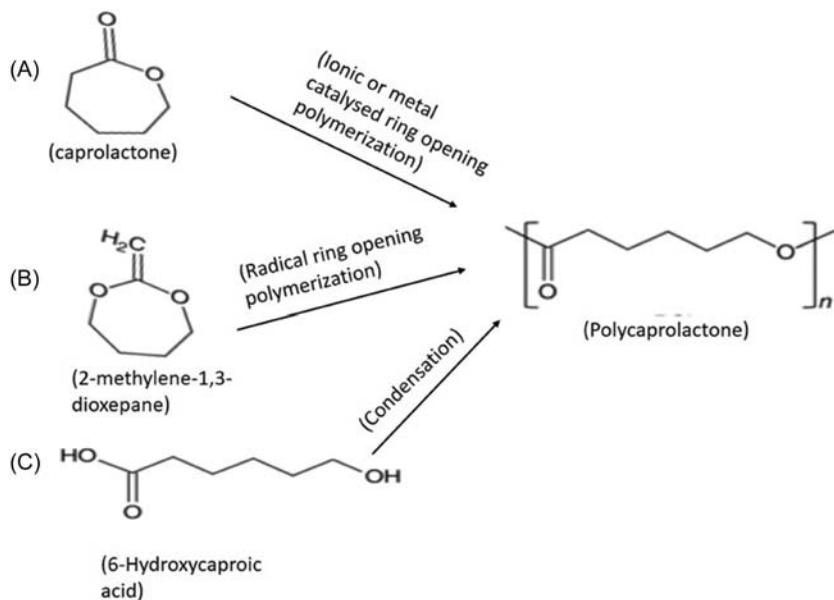


Figure 5.5. Different routes of formation of polycaprolactone from (A) caprolactone, (B) 2-methylene-1,3-dioxepane and (C) 6-hydroxycaproic acid.

nature generally increases with the decrease in the molecular weight of the polymer. It is generally used in numerous biomedical applications, such as scaffold printing and 3D-printed tablets to study drug delivery systems. Due to specific mechanical, physiochemical, and viscoelasticity properties, it is widely used in the production of variable shapes to study their degradation kinetics (Christen et al., 2020).

GelatinMethacryloyl (GelMA) is an example of a natural biomaterial that is derived from chemically modified denatured collagen. The chemical modification of the collagen is generally done by the addition of methacrylate moiety. Its photocrosslinkable property makes it useful in photo-patterning techniques to provide specific topography to the 3D scaffold. Fan et al. (2012) used two-step photopatterning process to develop 3D microgels from GelMA to capture neurons from microconstructs. In the first step, a photomask was used to give the shape of the prepared GelMA hydrogels (Fig. 5.6). These micropatterned hydrogels were immersed in GelMA solutions. In the second step, individual cells were captured at the gap position for photopolymerization (Yue et al., 2015).

Nanocellulose (NC), that is, nanostructured cellulose can be extracted either from plant cellulose in the form of cellulose nanocrystals (CNC), cellulose nanofibers (CNF), or from bacterial species (*Gluconacetobacter xylinus*) as bacterial nanocellulose (Fig. 5.7). All the three types are similar in their chemical composition; however, show difference in morphology, particle size, or crystallinity (Phanthong et al., 2018). Crystal and fiber forms of nanocellulose with specific characteristics like strength, surface area, and desired surface modifications according to the need

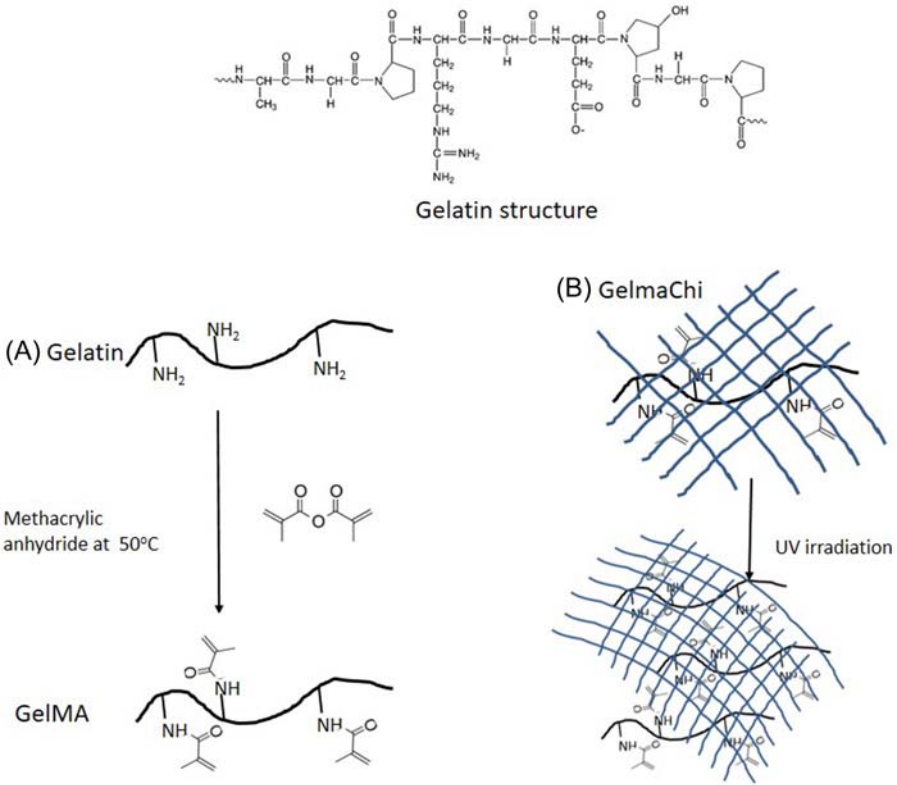


Figure 5.6. Scheme of formation of gelatin methacryloyl from gelatin.

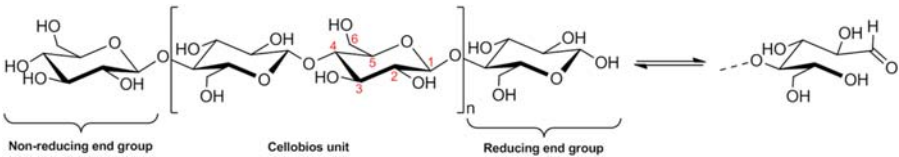


Figure 5.7. Structure of cellulose showing its units.

for stable and sustainable products can be developed through various chemical, physical, enzymatic, or combinatorial approaches (Trache et al., 2020).

The hydroxyl groups present in the unit structure of cellulose are responsible for the strong network of intra and intermolecular hydrogen bonding, which in turn provides physical and mechanical properties to the NC. The packing of units determines the crystalline (highly ordered region) and amorphous nature (disordered regions). CNF is a highly biodegradable material with negligible shear viscosity and high shear-thinning property makes its hydrogels suitable for 3D printing.

Role of three-dimensional printing in drug delivery

3D printing is defined as a sort of additive engineering that comprises the production of a 3D structure by dispensing or binding the materials in consecutive layers (Chua et al., 2020). Additive engineering or manufacturing, which is sometimes considered scalable manufacturing, is a subcategory of rapid prototyping that incorporates the methods of swift construction of prototypes and models (Günther et al., 2014). Some of the main additive manufacturing techniques used in the development of different pharmaceutical dosage forms are summarized in Fig. 5.8. There has been steady growth in the applications of 3D printing and different additive engineering techniques over the past 30 years in biomedical research; whereas, the healthcare and pharmaceutical industries are also evident with the increasing usage of such technologies in the past 15 years (Pham & Dimov, 2012). The mechanism of 3D printing is supported by an exceptional Computer-Aided Design and Draft software to construct the design of drugs or pharmaceutical products. Using this software, the design of a drug can be carved with standard features, such as printing speed, infill percentage, platform, extrusion temperature, etc. These parameters are followed by the 3D printers as instructions to formulate a drug product (Khatri et al., 2018; Norman et al., 2017). The raw materials used in the 3D manufacturing of drugs can be processed into filaments, granules, or binder solutions based on the acquired techniques, later to which the printed products are subjected either to drying, polishing, and sintering (Jain et al., 2018). These techniques are observed to

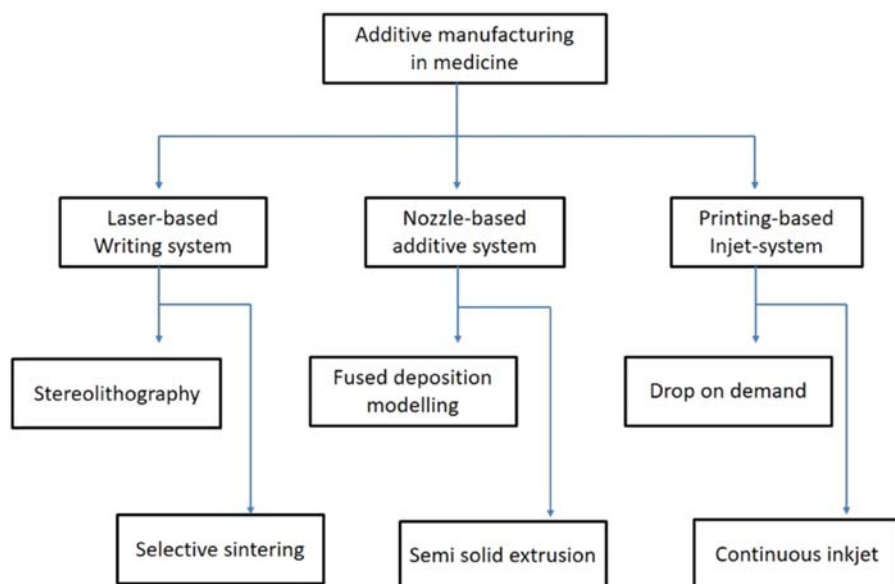


Figure 5.8. Three-dimensional printing techniques used for the manufacture of different forms pharmaceutical doses.

have a significant role in the R&D in different healthcare and pharmaceutical industries; also, they are being applied in the hospitals for the treatment of patients. The following section discusses the mechanisms of personalized drug delivery, which will include different methods of 3D printing.

Fused deposition modeling

Polymers such as PLA and PVA, which are thermoplastic in nature, are best in application with FDM processes of drug manufacturing (Emami et al., 2019; Piacentini et al., 2020). These polymers are commercially available as preprocessed filaments and are easily fed in the FDM systems in coiled form; thus, this bioprinting technique is also referred to as fused filament modeling. In this method of bioprinting, the filaments are fed through the rollers, and the liquefiers melt them to extrude out via the nozzle tip. These extruded materials are then allowed to cool and are subjected to solidification. To introduce flexibility in these bioprinted materials, an additional apparatus of hot melt extruder (HME) can be attached above the nozzle of the printer (Prasad & Smyth, 2016; Prasad et al., 2016). Customized formulations such as amorphous solid dispersions, which are used as raw material, can be prepared using the above configuration. However, with the incorporation of a mini extruder just above the nozzle tip via PED (precision extrusion deposition), the FDM process can accept granules or powder over filaments in the form of feed (Prasad & Smyth, 2016). Though several pieces of evidence indicate the use of PED in structuring the tissue scaffolds, they have not yet found applications in pharmaceutical preparations. However, there are few such instances that can lead to these prospected achievements, as the application of FDM is expanded to the deposition of bioinks, which can be given a shape to generate a final structure of any drug or therapeutic (Jamróz et al., 2018).

Extrusion-based bioprinting

Extrusion-based bioprinting relies on applying pneumatic or mechanical pressure to eject or dispense bioink in a simulated pattern through a nozzle (Panwar & Tan, 2016). The extrusion process is continuous in a layer-by-layer pattern until the desired shape and size of the drug are formed. The bioinks used in 3D printing are the biomaterials that comprise cells and other such biological entities. This bioprinting technique is highly applicable in the generation of the skeletal muscle (Fedorovich et al., 2007), aortic valve (Duan et al., 2013), bones (Phillippi et al., 2008), cartilage (Markstedt et al., 2015), and neuronal tissues (Hsieh et al., 2015), etc. However, the complications like mechanical strength and selection of the material are major concerns associated with bioprinting methods. The construction of vascular networks within the complex tissues is an unreciprocated question that the bioprinting technology is addressing even in the

current scenario; however, the concept of incorporating a sacrificial material within the 3D-printed construct can be used to create vascular channels in them (Suntornnond et al., 2017).

Drop-on-demand printing

As the name suggests, drop-on-demand (DOD) printing employs a more accurate operation with a lesser scope of waste generation. These bioprinters are equipped with nozzles that count to a value of 1000. The printheads used in this bioprinting technique are primarily induced by piezoelectric and thermal triggered mechanisms. The piezoelectric printheads are associated with the materials that are piezoelectric that fluctuate their volume in accordance with the applied electric current (Tekin et al., 2008). Since these materials are characterized to expand and contract under electrical induction, these surficial variations can generate adequate pressure to release or dispense a droplet at a shear rate of approximately 100–105/seconds (Goole & Amighi, 2016; Yun et al., 2009). On the other hand, the thermal print-heads employ the induction of electric current to produce heat in the volatile material (material to be printed) to generate a bubble which displaces a small volume of bioink out through the nozzle to form a drop. However, the application of thermal-based-DOD printing generates higher temperatures and uses bioinks with higher vapor pressure; therefore, the selection of attributes for preparing bioink must be considered important to avoid the disruption of heat-sensitive bioactive entities (Acosta-Vélez & Wu, 2016; Alomari et al., 2015).

Continuous jet printing

This printing technique involves the production or release of a constant stream of polarized droplets with an effect of pressurized flow. The charged or polarized droplets are driven over the substrate in an electric field generated between electrostatic plates, where they get deposited. This printing is quite efficient in its operations as the generated waste is recirculated to be used for further operations (Acosta-Vélez & Wu, 2016; Derby, 2010). To regulate the characteristics of drop formation and the bioink viscosity, both Continuous Jet and DOD printing employs the embedded printer head, which is either piezoelectric or thermal in function (Jain et al., 2018).

Stereolithography

Stereolithography, also called a laser-based writing system, was designed in 1980 by Charles Hullin (Ventola, 2014) and was commercialized in the year of 1986 (Jain et al., 2018). This bioprinting mechanism is based on photopolymerization, in which the free radicals are released due to the interaction of the photoinitiator with

UV light (Goole & Amighi, 2016). In stereolithography apparatus (SLA), the UV exposure induces explicit surface areas of photosensitive liquid resins to experience controlled polymerization (Carts, 1990; Neckers, 1990). The UV light transverses each of the 2D planes of liquid resin (i.e., x/y axis) while the SLA increments the z -axis to add to the volume of the drug (Ventola, 2014). Thus, the layer-on-layer addition of resins marks the synthesis of pharmaceutical products (Goole & Amighi, 2016). After the removal of the scaffold for the preparation of the structure, the finished structure exhibits surficial roughness, which can be toned by the application of edible coatings, sealants, or other primers (Jain et al., 2018).

Three-dimensional printing and drug delivery systems

Three-dimensional printing of hydrogels and emulsions for oral delivery

Hydrogels are the networks of cross-linking, biocompatible and hydrophilic polymers that dilate in aqueous conditions because of their compatible thermodynamic properties (Khodaverdi et al., 2019; Peppas et al., 2004). These 3D polymeric structures have found significance in various medical applications, such as biosensors, contact lenses, and materials for drug delivery carriers and tissue engineering (Peppas et al., 2004; Sri et al., 2012). The hydrogels are largely reported to effectively and conveniently administer protein drugs. Carrillo-Conde et al. (2015) prepared the hydrogel systems of P(MAA-*co*-NVP) and P(MAA-*g*-EG) for monoclonal antibodies (mAb) such as tumor necrosis factor (TNF). This hydrogel system is found to protect the TNF from the acidic pH of the stomach so that it can be released at the targeted site in the small intestine. Thus, these systems are conjectured to preserve the bioactivity of the antibodies, which leads to their intact circulation at the site of interest. Among the above-discussed hydrogel systems, P(MAA-*g*-EG) has appeared to be a promising candidate for oral delivery of therapeutic antibodies, protein drugs, and vaccines. The P(MAA-*g*-EG) hydrogels were demonstrated to be applicable for oral delivery of cholera toxins and vaccines (Yoshida et al., 2017). Another hydrogel based on alginate and loaded with BSA was developed as an ideal therapeutic (Lima et al., 2018). This hydrogel structure exhibited improved pharmacological activity and reported its pH-based swelling performance, which was highest at 7.4 pH. Likewise, poly-(*N*-vinyl imidazole) or xanthan gum hydrogel system, which is loaded with BSA, also exhibits high encapsulation and loading efficiency (Sabaa et al., 2019).

The emulsion is defined as a well-blended mixture in which two nonmiscible liquids, such as oil and water, are mixed with the catalytic activity of surface-active or emulsifying agents (Feas et al., 2017). Different emulsions such as water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) have found applications in controlled or delayed drug release (Khalil et al., 2013). The W/O/W emulsion techniques are reported for their effective entrapment of hydrophilic drugs in their internal aqueous compartment and, thus, are widely studied for their applications in protein encapsulation. However, this emulsion technique faces a broad disadvantages, including difficulties in controlling the size,

inconsistency with heat, pH and storage, and reduced activity of the protein (Koga et al., 2010). The W/O/W emulsions can be lyophilized to form ARMs (anhydrous reversed micelles), which respectively contain phosphatidylcholine and insulin in the oil and aqueous phase (Wang et al., 2010). This system was found to be effective in diabetic rats, where ARMs released insulin in a controlled manner, and the plasma glucose levels were significantly reduced. Another emulsion-based drug delivery system, namely, self-nano emulsifying drug delivery systems, received significant recognition as a potential alternative for oral administration because of its higher physical constancy and easy manufacturing (Campos et al., 2012; Maher et al., 2009). Apart from these advances, the application of emulsion electrospinning methods has led to the development of pH-sensitive nanofibers loaded with therapeutic proteins or drugs, which exhibit pH-dependent release of drugs and improves the stability of protein with a longer shelf-life (Panwar & Tan, 2016).

Three-dimensional printing of nanogels for nasal delivery

The nanogels are water-puffed, cross-linking polymers of nanoparticles of hydrodynamic size in the range of 10–100 nm and are dispersible in an aqueous medium while maintaining their fixed conformation (Mohtashamian & Boddohi, 2017; Soni et al., 2016). These nanogels can be synthesized either by using the combinations of natural and synthetic polymers or by using them individually (Aderibigbe & Naki, 2018). The characteristic parameters associated with nanogels, such as their size, network density, functional groups, and surface charge, can be regulated and tailored to achieve the intended functional and structural properties in drugs. It has been found that in comparison to free insulin, the nanogel-bound-insulin can easily cross the BBB to provide better neuroprotection against the A β -induced dysfunction. Nanogels are soft, multifunctional, and extremely hydrophilic in nature, which were found to be based on the poly-(N-vinyl pyrrolidone) and were covalently attached to the insulin (Picone et al., 2016). These findings strongly conjecture the application of nanogels as a promising tool in combating neurodegenerative diseases by the development of novel therapies. In another development, a system for intranasal vaccine delivery was developed by Nochi et al. (2010) that consisted of a nanogel-cationic cholesteryl-group-bearing pullulan (cCHP), which was supposed to intranasally administer a neurotoxin-BoHc/A, an anon-toxic fragment of type-A *Clostridium botulinum*. This neurotoxin was taken up by the mucosal dendritic cells after it was released from the cCHP nanogel. In the same research, the cCHP nanogel has been reported as an efficient vehicle for adjuvant-free, protein-based-antigen, which triggers stronger mucosal-specific and systemic immune response on being intranasally administered by the tetanus toxoids.

Three-dimensional printing of hydrogels for rectal delivery

The hydrogel formulations are effective in enhancing the bioavailability of protein therapeutics through the rectal mode of administration. A novel hydroxyl propyl

methyl cellulose (HPMC)-*co*-PAM-*co*-PMAA hydrogel (hydroxypropyl methyl cellulose-*co*-polyacrylamide-*co*-methacrylic acid) was developed by [Shi et al. \(2019\)](#) to be given as a rectal suppository. This hydrogel was furnished by the method of free-radical polymerization, which was occupied with insulin to manage the levels of blood glucose in patients with diabetic conditions. This hydrogel was reported to be noncytotoxic in nature and was able to release insulin consistently at 7.4 pH (in the rectal environment) and was also reported for its *in vivo* hypoglycaemic effect. Another innovative binary hydrogel was developed by [Xue et al. \(2018\)](#), which also contained insulin through solution polymerization; it exhibited reduced levels of blood glucose in hyperglycaemic rats by releasing the insulin in a sustained manner. The micropores of these binary hydrogels can lodge a higher concentration of intended drug molecules. These applications of 3D-printed hydrogels confer vital preclinical observations for an effective rectal therapeutic delivery system of protein drugs.

Three-dimensional printing of patches and microneedles for transdermal drug delivery

3D printing technology modifies the drug delivery systems for skins, as a result more sophisticated and precise transdermal drug delivery systems are explored by the researchers. The main aim of transdermal drug delivery is to the inner layer of skin through which drug molecules will pass to the blood circulation. For this, various materials are used to develop transdermal patches, microneedles, etc. In the past, medicines containing drug molecules were directly applied to the skin and with time they absorb within the stratum corneum. However, with time this therapy is shifted to act by slow channeling of drug material to the epidermis.

Transdermal microneedles are miniature devices that are used for the transfer of drug molecules vaccines or even genetic materials like DNA or RNA to the inner layer of skins. These microneedles can create micropore in the skins through which desired drug molecules can be channelized into the dermis. Through this technique, minimum invasive along with improved drug response and bioavailability is reported ([Pere et al., 2018](#)). After optimizing various parameters of this drug delivery system it showed desired mechanical strength and piercing capacity proving itself as a suitable transdermal drug delivery system ([Yu et al., 2017](#)).

Initially, microneedles were prepared by the micromoulding method, which was a time-consuming process and difficult to scale up. In addition, accuracy, reproducibility, drug deposition, and limited loading of active components are some of the main drawbacks that are needed to be resolved for the betterment of this drug delivery system. [Pere et al. \(2018\)](#) used photopolymerization-based 3D printing, a technique for the fabrication of microneedles structures. Here, they used UV-sensitive polymers layer-wise polymerization to print insulin-coated microneedles in pyramid and cone designs. Furthermore, they analyze the release studies of the coated insulin in the abdominal porcine skin (Franz diffusion cells). SEM images of various 3D-printed microneedle are summarized in [Fig. 5.9](#).

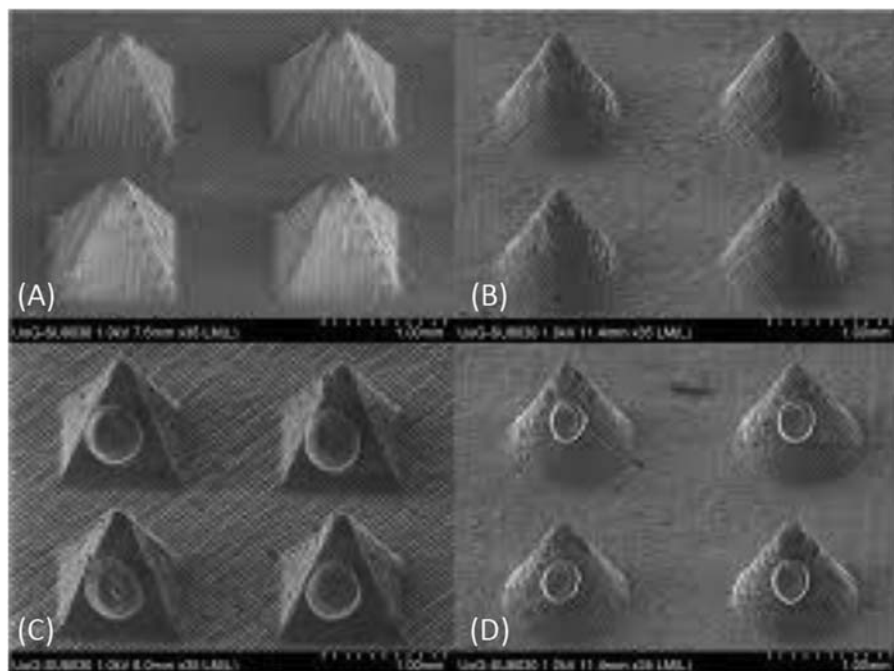


Figure 5.9. SEM images of three-dimensional printed microneedle designs of (A) plain pyramid, (B) plain cone, (C) insulin coated pyramid, and (D) insulin coated cone.

Source: Reproduced with permission Pere, C. P. P., Economidou, S. N., Lall, G., Ziraud, C., Boateng, J. S., Alexander, B. D., . . . Douroumis, D. (2018). 3D printed microneedles for insulin skin delivery. *International Journal of Pharmaceutics*, 544(2), 425–432.

Caudill et al. (2021) designed and fabricated a faceted microneedle using the continuous liquid interface production technique of 3D printing to evolve an advanced approach toward the vaccines developments containing multiple components. In this approach, 3D-printed co-loaded vaccine model shows an efficient delivery of its components and supports in maintaining the humoral and cellular immunity also. This initiation provides a direction to improve vaccination procedures for better healthcare.

Three-dimensional printing of tablets for oral delivery

Oral drug delivery is one of the most common paths of drug delivery. There are a number of release profiles used according to the need of the patient, which resulted in the development of tablets with various forms of the drug release patterns. Some of these are summarized in Table 5.3.

Table 5.3 list of different drug release pattern used in the development of 3D printed tablets.

S. No.	Tablet	Model Drug	materials	Techniques	References
1.	Channeled tablets	Hydrochlorothiazide	polymers	FDM	Sadia et al. (2018)
2.	Immediate Release Tablets	pantoprazole sodium	Polyvinylpyrrolidone, polyethylene glycol, Kollidon and poloxamer were used	FDM/HME	Kempin et al. (2018)
3.	Immediate Release Tablets with liquid crystal-forming drug	itraconazole	polyvinylpyrrolidone-based polymers	FDM/HME	Jamróz et al. (2020)
4.	Immediate Release Tablets for a poorly water-soluble drug	lumefantrine	butylated methacrylate copolymer, xylitol, maltodextrin	FDM	Fanous et al. (2021)
5.	Delayed Release Tablets	Paracetamol	Hypromellose acetate succinate, methylparaben	FDM/HME	Goyanes et al. (2017)
6.	intragastric floating and sustained-release tablet	Venlafaxine hydrochloride	hydroxypropyl methylcellulose for core and polylactic acid for an insoluble shell with air chamber	FDM/HME	Zhao et al. (2022)
7.	Pulsatile Drug Release Tablets	Diclofenac	Eudragit	Inkjet	Siamidi et al. (2020)
8.	Bilayer tablet	Guaifenesin	Hydroxypropyl methylcellulose, poly(acrylic acid), microcrystalline cellulose, sodium starch glycolate	Extrusion	Siamidi et al. (2020)
9.	multiple Release Tablets	Paracetamol	Polyvinyl Alcohol	FDM	Xu et al. (2019)
10.	Controlled release tablet	fenofibrate as the drug	Beeswax	FDM/HME	Kyobula et al. (2017)

FDM, Fused deposition modeling; HME, hot-melt extrusion

Immediate release tablets

When there is an immediate need for medication then this form of tablet is very useful. This type of tablet is prepared by combining the drug candidate with the hydrophilic polymer like hydroxyl propyl cellulose (HPC), HPMC, etc. [Bhatt et al. \(2021\)](#) used the fused deposition modeling along with hot melted extrusion method for 3D printing of immediate-release tablet using olanzapine as a drug candidate.

Pulsatile drug release tablets

For several diseases like bronchial asthma, rheumatoid arthritis, ulcers, etc, the circadian cycle of the body plays an important role, that is, patients require a drug dose at a specific time. In this case, this form of a tablet is very effective by providing a well-timed pharmacological effect to the patient, this, in turn, reduces the side effect than arises through sustained drug exposure ([Jain et al., 2011](#)). [Dumpa et al. \(2020\)](#) used hot-melt extrusion technology along with FDM 3D printing method for development of HPC and ethyl cellulose (EC)-based filaments to develop novel core-shell gastroretentive floating pulsatile drug delivery systems.

Monolithic sustained-release tablets

The requirement of multiple doses per day of any drug is one of the problems concerning patients. To overcome this problem, researchers developed various other forms of drug systems with controlled release pattern. [Kong et al. \(2018\)](#) developed a sustained-release tablet of nimodipine drug, which is poorly soluble in water using monolithic osmotic pump technology. FDM 3D-printed sustained release tablets were prepared by loading the drug candidate with commercially available matrix-like PVA (filaments)

Biphasic release tablets

Whenever there is a need for a rapid therapeutic response and then a prolonged release at a certain concentration is required to avoid the repetitive administration of medication, fast/slow biphasic release systems are recommended. [Khaled et al. \(2014\)](#) reported 3D printing of a monolithic sustained release tablet containing naproxen as a drug candidate. In this experiment, HMC with poly(acrylic acid) as a hydrophilic matrix for the formation of a sustained release layer while microcrystalline cellulose with sodium starch glycolate was used for the immediate release layer. This reduces the hardness of the tablet as compared to the marketed product.

Channeled tablets

Use of polymers in the printing of tablets through FDM 3D-printed technique slows down the release of drugs. To minimize this drawback of the 3D printing technique, [Sadia et al. \(2018\)](#) developed an innovative approach using hydrochlorothiazide as

a model drug and designed a tablet with perforating channels of variable width, length, and alignment, which in turn increases the surface area/volume ratio, which accelerates the drug release from 3D-printed tablets. They also suggest the length of channels as an important criterion and prove in their work that the multiple channels shorter in length are better than longer ones, despite having comparable surface area/mass ratio.

Conclusion

Modern lifestyle is one of the key factors for the emergence of various health conditions. To combat these health concerns, we need to develop more precise medicines and nutraceuticals. Personalized medicine is supposed to provide more specific medical treatment to the patient after properly analyzing the general and genetic features of the patients along with their lifestyle to get an idea of the epigenetic role in the development of disease, which has been the center of attention of the researchers over the recent years. 3D bioprinting is the most promising technique for the development of personalized medicines and nutraceuticals. Although this technique is in its emerging phase, scientists are experimenting with its ability in the diverse area according to the need. FDM, hot-melt extrusion, stereolithography are some of the most commonly used 3D printing techniques used in the development of more effective drugs or implants. This technique can play a promising role in the revolution of the pharmaceutical sector in the near future.

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Application of 3D printing in food industry

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Introduction

Three-dimensional (3D) printing or additive manufacturing can be used to fabricate a wide array of complex structures and architectures by printing layers of material on top of each other. It was first developed by Charles Hull in 1986 by stereolithography. 3D printing has advanced to various principles and now is being used for different end-use applications. The evolution of 3D printing can drastically change the manufacturing processes. At present, 3D printing is not only limited to automobile, construction, or manufacturing industries but also has expanded to other biological sectors, including food, agriculture, drug delivery, and biomechanical processes (Stansbury & Idacavage, 2016). While traditional manufacturing can be efficiently used for mass production of identical parts, 3D printing has proved to be helpful for low-volume production of complex structures with minimal material wastes (MacDonald & Wicker, 2016). New applications are being developed continuously as research advances in this field.

Printing in 2D form in food sector has been practiced before 3D printing, for example, printing text/image/logo on edible products, such as cakes and cookies using ink jet sprays. 3D printing in food was first introduced by researchers from Cornell University, Fab@Home (Nachal et al., 2019). It was an extrusion-based printer, capable of printing a wide variety of shapes from different print materials. Currently, two major companies—Stratasys and 3D Systems—are majorly involved in production of chocolate-based 3D-printed items. 3D System's ChefJet series prints products from powdered sugar and cocoa using Z-Corp inkjet process. Other examples include Foodform 3D by RIG, Foodjet by De Grood Innovations, Foodini

by Natural Machines, and CocoJet by 3D Systems (Lipton et al., 2015; Nachal et al., 2019). 3D printing for food sector is different from robotics-based food manufacturing in which latter case replicates only the manual processes in food structuring. 3D printing relies on phase transitions or chemical reactions for fabrication of cohesive fused forms. 3D printing has paved the way for customized design, shape, flavor, and nutritional components according to the consumer need (Wegrzyn et al., 2012).

Food three-dimensional printing process

The food printing process can be majorly categorized in three steps, Fig. 6.1 describes these steps in a diagrammatic way. First, a 3D CAD model is designed of the required product. This design can be created or scanned from a model to know about surface features and the required geometry. Second, a suitable splicing software is used to splice the model in different layers for the printing command. During this process, G-codes and M-codes are generated that guide the motors for proper printing of the model. The third step is the posttreatment applied to the printed food (Guo et al., 2019). The existing 3D printers can be categorized in seven types on the basis of feed intake, the type of energy applied on ink, and number of materials dispensing capability. These are namely binder jetting, material jetting, extrusion, sheet lamination, powder bed fusion, directed energy deposition, and vat polymerization. Each of them can be categorized further into subclasses (Lille et al., 2018). A detailed process of printing technologies has been discussed in Chapter 2. In the present chapter, we discuss about the models of 3D printers in food.

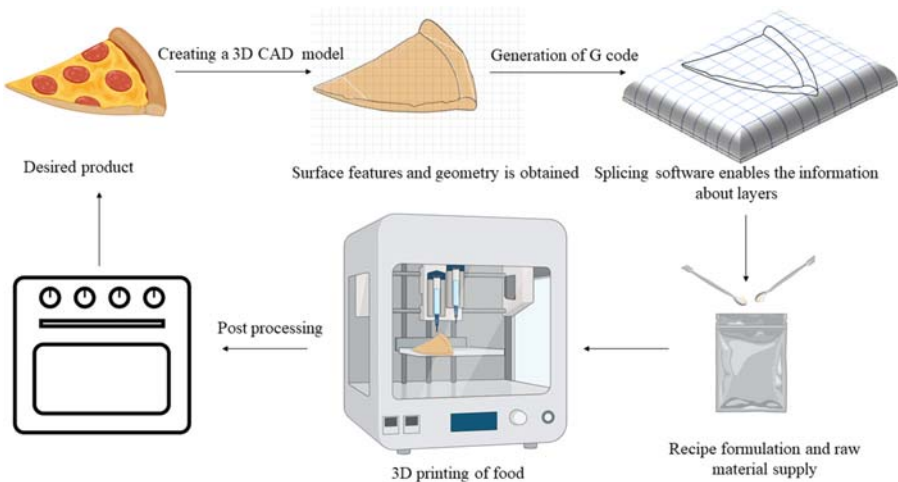


Figure 6.1 Process of three-dimensional printing of food.

For 3D printing of food, extrusion is the most common method for printing. The main advantage of using extrusion method is the ease of use and the availability of different foods in gel form that can be extruded to form an entire meal. A number of food materials have been printed using extrusion-based 3D printing, which has been discussed later in this chapter. Extrusion method is based on fused deposition modeling where a fixed/movable nozzle moves over a movable/fixed plate in patterned layers. These layers are stacked over each other to form a 3D object (Lanaro et al., 2017). The major limitation with extrusion is that it is very difficult to obtain a smooth surface and create angles with sharp overhangs. Food gels tend to collapse at overhangs.

Another common approach is hot air/laser sintering method where hot air/laser can be used as heating element to fuse the powder particles and form a solid layer. This approach is generally used to print fat-based or sugar material whose melting point is relatively low. The printing platform is heated just below the melting point of the material to facilitate the fusion with the next layer. This process does not require postcuring process (Sun et al., 2015). Inkjet printing technique is used for printing of bakery items, such as cookie, pastry, or cakes where the nozzle prints in a drop-on-demand manner. Alternatively, binder jetting method is also used for printing of sugar material where a powder component and an “ink” (binder) is printed in layers. The ink/binder causes agglomeration (physical or chemical) of the powder material. In this approach, it is easier to incorporate multimaterial component and also to form complex geometry shapes, which is challenging in extrusion method (Holland et al., 2019).

Advantages of three-dimensional-printed food

3D printing can provide a major breakthrough in our understanding of food processing and the delicate relationship between the food chemistry, machinery, and palatability. It can also pave the way for new food products with additional nutritional value, color, texture, and flavors. With the rise of chronic diseases, there has been a growing interest in nutritious foods that can provide antioxidants, vitamins, and other bioactive compounds to combat against these diseases. 3D printing provides an alternative solution by incorporating specific nutrients in the printed food. It can also pave the way for personalized food as per the nutritional requirement of the person (Yang et al., 2017). At Cornell Creative Machines Lab, they have printed two cookies according to the nutritional requirement of individuals. The cookies texture was identical and was based on the calorie intake of different health histories. 3D printing can also be applied to places with limited access to food or in calamity. Inks can be developed with ingredients that have shelf life longer than conventional fruits and vegetables that can get spoilt after certain days. Eventually, 3D printing can be helpful in space missions where astronauts can have access to nutritious foods with relatively longer shelf life (Lin, 2015). 3D printing can also be used to print “soft food” for the elderly people who have difficulty in chewing. Food paste can be printed in a customized form for it to be palatable (Pulatsu et al., 2020).

Another advantage is the printing of complex geometry structures, which may not be possible in traditional cooking methods. It is time-saving, cost-effective as compared to the mold system and is customizable. The users have the access to a library of shapes and structures to choose from. Fig. 6.2 demonstrates different geometrical shapes developed from lemon juice gel. Lipton et al. have optimized the parameters and observed that 1 mm nozzle diameter, 24 mm³/second extrusion rate, and 30 mm/second nozzle movement speed were found to be the optimal parameters to print 3D constructs matching the target geometry with fine resolution, smoother surface texture, and fewer point defects with no compressed deformation (Yang et al., 2018).

Recently, there has been an increase in demand of personalized foods. However, these personalized foods are two-three times costlier than the traditional products, takes longer delivery time, and lower mass production. Zhou et al. were able to detect the salient features of the image using image extraction technique and were able to print images, such as a human face using materials such as maltose, chocolate sirup, jam to print customized patterns (Zhao et al., 2018).

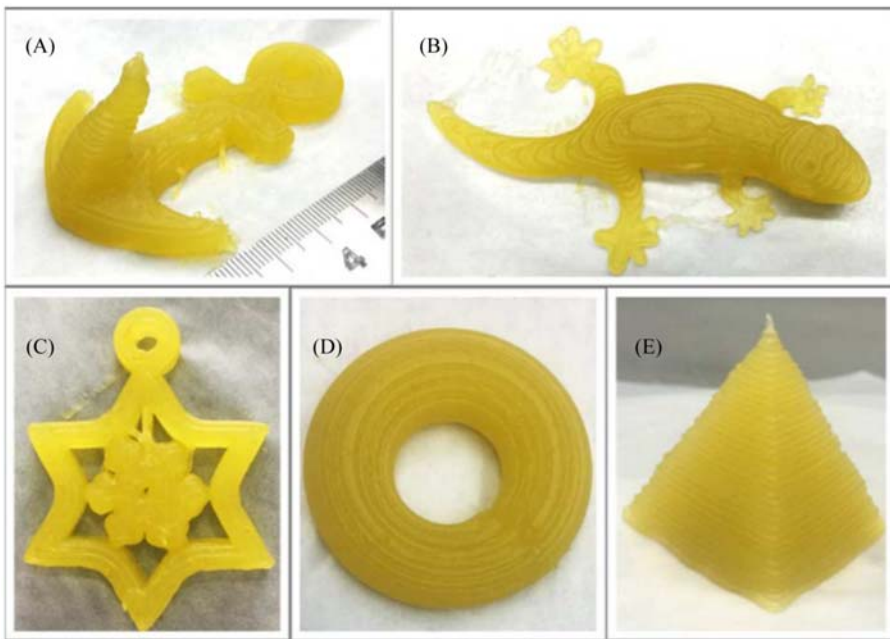


Figure 6.2 Pictures of some three-dimensional printed products printed at 24 mm³/s extruded rate, 30 mm/s nozzle moving speed and 1.0 mm nozzle diameter (A. Anchor, B. Gecko, C. Snowflake, D. Ring, E. Tetrahedron).

Source: Reproduced with permission from Yang, F., Zhang, M., Bhandari, B., Liu, Y. (2018). Investigation on lemon juice gel as food material for 3D printing and optimization of printing parameters. *LWT*, 87, 67–76.

Though the cost of 3D printer is high, there is a prediction that 3D printing of food at mass production may be able to increase the profits at retailer level. Jia et al. have studied the financial viability of supply chain for 3D-printed chocolates through modeling and simulation. They observed that if 3D printing of chocolates is done at manufacturer's end, the manufacturers are able to make more profits while the retailer profits tend to be stagnant. Alternatively, if retailers are using 3D printing technology for the customers, a potentially disruptive business model was predicted that may pose a challenge to traditional high-end chocolate products (Jia et al., 2016).

Three-dimensional-printed foods

Confectionary—chocolate and sugars

Chocolate is one of the most popular flavors worldwide. It has been consumed from a very long time and is believed to have been originated from Mexico where it was named as “a gift of the gods.” Chocolate is produced from cacao seeds and is primarily harvested in West Africa, Indonesia, and Sri Lanka but consumed all over the world with Europe and United States being the leading consumers in the world. Chocolate production starts by fermenting the seeds from the pods of cacao tree. The beans are then dried, roasted, and crushed and pressed into cakes. These cakes are then alkalized to form cocoa powder. This cocoa powder is then mixed with different proportions of sugar, fat, and sometimes milk to form different flavors of chocolate. Chocolate has been associated with health benefits and also with addiction. There are reports that suggests the presence of antioxidants that promote the cardiovascular health, whereas there are also reports of cravings and addiction. Although it is not classified as a substance of abuse but the high amount of sugar and fat does not categorize it as a “healthy” snack (Bruinsma & Taren, 1999; Serafini et al., 2003; Waterhouse et al., 1996).

Chocolate has been the first material to be 3D-printed and still being used to form different complex geometries. The major advantage for chocolate printing is that it easily melts at 50°C and can solidify at room temperature (De Graef et al., 2011). Melted chocolate exhibits non-Newtonian fluid and shear thinning behavior. This means that melted chocolate has constant viscosity independent of stress. A non-Newtonian fluid is generally preferred for extrusion-based 3D printing as the ink has to undergo stress through nozzle and also retain its shape after printing. This may also pose disadvantage as shear stress and the strain rate is nonlinear. A number of commercial printers are also available in the market specifically for 3D printing of chocolates (Jia et al., 2016).

However, despite the advantage of using chocolate as an ink and the consumer demand, it still remains a challenge to 3D print it. It requires a skilled workforce to design and print the 3D model of chocolate. Also, there are various kinds of chocolate in the market that differ upon its composition. Different types of chocolate can be prepared by using different ratio of butter, milk, cocoa, and a number of

different triglycerides. Using these triglycerides, different forms of chocolate can be obtained of varying thermodynamic stability (form I– VI), where type V or β is the most preferred form in the chocolate industry. It also known as tempered chocolate as it is tightly packed, stable, and has glossy appearance. This form is more preferable for chocolate printing because of its superior properties in terms of hardness and melting point (Afoakwa, 2016; Beckett, 2009, 2018).

Understanding the relationship between process chemistry and kinetics of extruded model will be helpful in better construct of 3D-printed model. Also, there should be a balance between the stress applied to chocolate through extruder nozzle and the cooling temperature required to maintain the shape stability of the 3D construct and the ability to hold its own weight. Lanaro et al. attempted to 3D print chocolate in the form of complex structures. They have used melt extrusion method for printing and optimizing various parameters to print a 3D model without collapsing. As depicted in Fig. 6.3, they have printed a chocolate bunny with steep build angles and with overhanging parts. The two parameters that need to be optimized are—designing of the extruder assembly and the cooling system. The extruder assembly needs to be as rigid as possible, thereby reducing flexion and enabling a more accurate deposition of chocolate to form layers. As the ink gets extruded, the material should be quenched using a cooling system so that the shape stability is maintained. Quenching the printed product with a temperature difference of 3.8°C was able to form self-supporting layers. The movement speed had negligible effect on forming self-supporting layers (Lanaro et al., 2017).

Process optimization was also attempted by Hao et al. They used optimized various process parameters like extrusion rate, nozzle velocity, and nozzle height, which were critical for a 3D-printed product. They observed that 1.25-mm nozzle

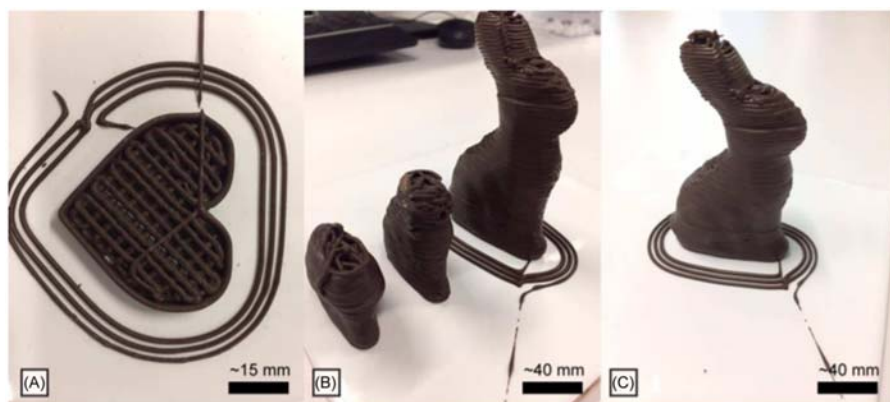


Figure 6.3 Three-dimensional-printed chocolate. (A) Shape of heart (height is 9 mm), (B) chocolate bunny of height 30 mm and 42 mm, and (C) chocolate bunny of height 94 mm. *Source:* Reproduced with permission from Lanaro, M., Forrestal, D.P., Scheurer, S., Slinger, D.J., Liao, S., Powell, S.K., Woodruff, M.A. (2017). 3D printing complex chocolate objects: Platform design, optimization and evaluation. *Journal of Food Engineering*, 215, 13–22.

aperture size and 2.9-mm nozzle height were the optimized parameters for a good printability. The “line test” revealed that the bead diameter increased in proportion to the extrusion rate (Hao et al., 2010). Most of the studies based on chocolate 3D printing was based on hot extrusion as it is easier to melt and control the rheology at higher temperature. Karyappa et al. used cold extrusion method for chocolate 3D printing at room temperature. Cold extrusion process solely depends upon the rheology of the ink to be extruded. They have used cocoa powder as additive of different concentrations in chocolate sirup and paste to obtain ink of different rheology. They observed that an increase in cocoa powder in the ink exhibited shear-thinning properties. They optimized the printing parameters, such as nozzle pressure, printing speed, and the distance between two layers, and were able to retain the shape stability of the 3D model without increasing the temperature (Karyappa & Hashimoto, 2019).

Chocolate can also be altered for its rheology and flow properties. The printed material should have a smooth texture and glossy appearance for a good printability. The composition such as fat content and type of emulsifier and the form of chocolate decides the ultimate outcome. During extrusion method of printing, there is slippage in the extrusion tube that hinders the flowability from the nozzle. As such, the model may not have a smooth structure. To overcome this, some additives may be added to chocolate that refines its rheology but does not affect the taste of the printed model. Mantihal et al. observed that the addition of magnesium stearate and plant sterols were able to improve the rheology of chocolate through an auger type extruder. The additives did not alter the thermal property and retained the pseudoplastic behavior of traditional chocolate (Mantihal et al. 2017, 2019). There still exists a major research gap for commercialization of 3D-printed chocolate like optimizing the physical parameters of 3D printer, the composition of the chocolate, the viscosity, rheology, and the tribology characteristics of the printed model. The printers can also be designed in a way for rapid, cheap, and the ability to print complex models.

A lot of research has been focussed on printing of complex structures using sugars. The major advantage of printing sugar is that it does not require postprocessing treatment and the product can be used directly. The earliest example of 3D printing includes printing of a “cake mix” by extruding a mixture of sugar, corn sirup, starch, yeast, and cake frosting. Later laser beam was used to melt and a binding agent was used to fuse the granular materials. This granular bed sintering technology was adapted for printing 3D models by sucrose using hot air melting technology. Candyfab.Org has been using this technology to develop sugar-based complex structures. 3D printing of sugar has come a long way since with more complex structures being commercially available for the consumers (Lille et al., 2018; Sun et al., 2015).

Dairy products

Food 3D printing has been exploring a lot of materials to be printed—chocolate, cookies, pizza, meat but very few research is focussed on 3D printing of dairy

products. There is a large scope in optimizing the printing parameters for milk-based products. Milk and other dairy products like cheese and yogurt are consumed all over the world and forms an integral part of the daily diet. They are a rich source of calcium and other nutrients that has health promoting potential (Acham et al., 2020). Milk is an emulsion or colloid system consisting of lactose, whey protein, and salts or minerals as continuous aqueous phase while fat globules and casein micelles in the dispersed phase. Whole milk contains 35–40 g/kg of fat in the form of water-in-oil emulsion (globules) surrounded by lipoprotein membrane.

Milk protein such as milk protein concentrate and whey protein can potentially be used as food ink since they have the property of heat induction and gelation (Jost, 2000; Nguyen et al., 2017). This property can be utilized for extrusion-based 3D printing method where the viscosity of the material is an important factor. The material should be thick enough to extrude through the nozzle and can adhere to other layers. Both milk protein isolate and whey protein are nutritious in nature and are now being consumed by athletes and fitness enthusiasts for high protein fiber intake (Ji et al., 2017). Liu et al. investigated that milk protein isolate and whey protein can be printed in the ratio of 5:2 to obtain a 3D model. The protein paste prepared in this ratio had the mechanical strength and viscosity required for extrusion process. They also observed that with an increase in whey protein concentration, there was an altered interaction between water and the protein as estimated by rheological behavior and NMR results (Liu et al., 2018). When printing high protein content for nutritional benefit, it is also important to have lower fat content or high fiber content. Since milk naturally has fat content, it can be mixed with fibers. This will not only improve the nutritional property of the printed food but also the mechanical strength. Lille et al. were able to print semiskimmed milk powder-based paste using extrusion-based 3D printer. They were able to print protein based fibers, such as milk powder-, oat- and faba bean protein-based material with minimal fat content, thus enabling printing of nutritional food (Lille et al., 2018). The best printing model was obtained by 10% cold swelling starch, 15% skimmed milk powder, 60% semiskimmed milk powder, 30% rye bran, 35% oat protein concentrate, or 45% faba bean protein concentrate. For achieving shape stability high yield stress was required. A high solid content in the form of fibers was beneficial in retain the shape of 3D model even after oven drying.

Milk protein is a “soft material” and addition of certain materials are required to increase the rheological property as well as mechanical strength to create 3D models of precise geometries and surface features. This model should be strong enough to maintain its integrity and soft enough for consumption purpose. Liu et al. prepared a gel-like structure by dispensing milk protein concentrate into sodium caseinate solution. They observed that the protein gel with 400–450 g/L of total protein content was able to extrude and retain the shape of 3D model. They analyzed the rheological and textural properties of the ink and observed that with increasing total protein content, the inner structure of the milk protein gel transformed from a tangled state to a transient 3D network. An increase in total protein content also increased the apparent viscosity, thixotropy, and yield stress of the printed model (Liu et al., 2019). Apart from food printing, additive manufacturing

has also been applied to print an optofluidic microviscometer for the presence of any milk adulteration. The researchers were able to detect 1%–10% of adulterants in 60 samples of milk (Venkateswaran et al., 2016).

Cheese is produced by coagulation of milk protein casein and is consumed worldwide with different flavors and textures. Cheese contains high amount of fat and has longer shelf life than milk. Different types of cheese is consumed worldwide that depends upon the source of milk, fat content, pasteurization, coagulation method, and processing technique (Korhonen, 2009; Spreer, 1998). Traditional cheese-making process is very similar to the 3D printing process where the ingredients are mixed in a fixed proportion at a high temperature under vacuum and then casted into desired shapes for packaging. Le Tohic et al., were the first group to study the impact of additive manufacturing on structural properties of dairy products. The researchers have used extrusion method of 3D printing with printing speed of 4 mL/minute (low speed-printed cheese) and 12 mL/minute (high-speed printed cheese). The cheese used in this study was a commercially available processed cheese, containing 25% fat, 3% carbohydrate (of which 2% is lactose), 18% protein, and 3% salt. The effect of printing speed was compared with the untreated processed cheese and melted processed cheese but not extruded one. For processing, the melted cheese was heated at 75°C for 15 minutes while the printed cheese was heated for 12 minutes as it had to undergo shear stress during the printing process so that the melting characteristics are same for each sample. The textural analysis revealed that the printed cheese had significantly reduced hardness and significantly increased meltability. The compression-decompression cycles revealed that it required almost twice force for untreated than the printed cheese. This implies that the printed cheese had better texture than the untreated sample. The samples were observed under confocal microscope to observe the distribution of protein and fat. As depicted in Fig. 6.4A, the untreated sample had uniform distribution of fat globules (stained red) in protein phase (stained green) as is the characteristic of cheese. Melted cheese had larger fat globules than untreated as they tend to coalesce upon heating—Fig. 6.4B. For low speed-printed cheese, the protein phase appears discontinuous and larger nonspherical fat globules were observed while high speed-printed fat globules tend to be smaller with a more uniform size distribution as shown in Fig. 6.4D. Thus, extrusion through a nozzle has different effects on microstructure depending upon its intensity. Low shear facilitates coalescence by disrupting the protein phase and facilitating fat globules interactions whereas, in contrast, high shear appeared to have a greater effect upon the fat globules, reducing their size in comparison to lower shear extrusion. Therefore, the recipe should be optimized for minimum alteration in food microstructure (Le Tohic et al., 2018)

Meat

Meat has been a major source of nutrition in many parts of the world. The demand for meat is increasing and has now quadrupled in the past 50 years. Worldwide 320 million tonnes of meat is produced (Ritchie & Roser, 2020). By definition, meat

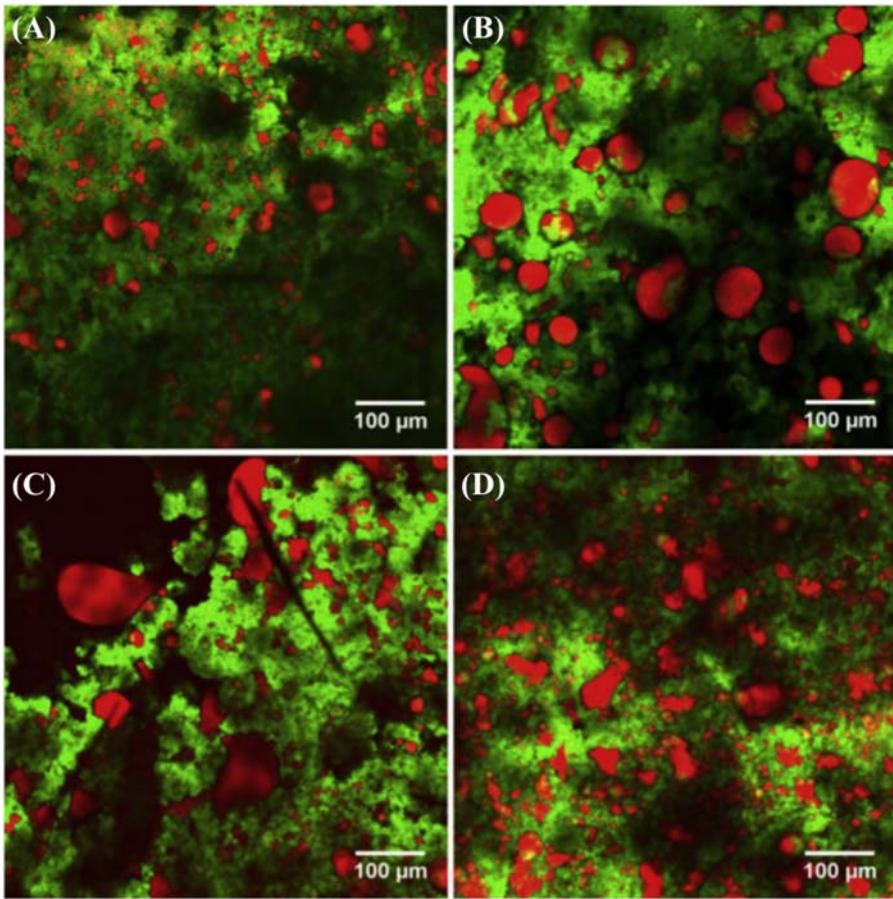


Figure 6.4 Cheese samples (A) untreated cheese, (B) melted cheese, (C) low speed processed cheese, and (D) high speed processed cheese stained with Nile Red (lipids) and Fast Green FCF (protein) solutions and observed under confocal microscope.

Source: Reproduced with permission from Le Tohic, C., O’Sullivan, J.J., Drapala, K.P., Chartrin, V., Chan, T., Morrison, A.P., . . . Kelly, A.L. (2018). Effect of 3D printing on the structure and textural properties of processed cheese. *Journal of Food Engineering*, 220, 56–64.

and seafood are nonnative printable materials. They are processed and consumed in traditional methods much like the fruits and vegetables. As a major composition of meat is fibrous tissues, which makes it difficult to have printability features. The major disadvantages with the printability of meat are—it does not have flow property like cheese or butter, it does not crystallize or harden by change in temperature after printing of the model, and it needs postprocessing—frying or baking before consumption of the printed model.

Although very few research has been dedicated towards the printing of meat products, these studies have certain additives that can enhance the rheological property of meat to be extruded through a nozzle. Lipton et al. have added transglutaminase and bacon fat in turkey meat to obtain the rheology of a paste. They have printed turkey meat using solid free form fabrication method and postprocessing was done by conventional cooking method. The printed 3D model was able to retain its shape and stability (Lipton et al., 2010). Liu et al. have used gelatin in chicken, pork, and fish to obtain a slurry-like composition. They were also able to maintain the nutritional quality of the meat making it easier to accomplish ketogenic diet (Liu et al., 2018). Similarly, Wang et al. used have added NaCl in fish surimi gel. They added different concentrations of NaCl (0%, 0.5%, 1%, 1.5%) to fresh silver carp fillet mince. The rheological properties were evaluated by water holding capacity, gel strength, and distribution of water content within the gel. The optimum concentration was obtained at 1.5% to obtain suitable printing property (Wang et al., 2018). However, the authors have not evaluated the postprocessing effect on the printed model.

3D printing of meat will be helpful for elderly people who have chewing problem and do not get any taste sensation. Pureed foods with desired shape and flavor by 3D printing can be advantageous for such people. Kouzani et al. printed a fish-shaped model using purees of tuna, pumpkin, and beetroot. They have used pressure-controlled extruder working at a temperature of 20°C (Kouzani et al., 2017). The processing temperature plays a major role in determining the material behavior, the flow properties and final stability of the model.

Another emerging method is the combination of 3D printing and tissue engineering. To reduce the burden of animal slaughtering, meat can be printed by culturing of stem cells derived from the animal. Though currently it is in naïve stage, but it is a promising technology for the future. In this method, stem cells from the animal are printed over an agarose gel support through an inkjet printer. The cells fuse together and form an engineered meat. This meat is then subjected to low frequency stimulation in a bioreactor to mature into meat fibers (Dick et al., 2019). However, there are still a number of challenges that need to be addressed before using this technology, such as sensory and nutritional profile of the printed meat, consumer acceptance, cost-effectiveness, and safety of the bioprinted food. In a study of consumer responses toward 3D-printed meat and insect-based foods, people have raised certain concerns over the acceptability of such meats. There were some terms such as “not fresh, potentially harmful, processed, lacking taste or not nutritious.” These challenges have to be addressed before using the full potential of 3D printing technology (Lupton & Turner, 2018).

Bread and bakery

Unlike candies and sugar, bakery items such as cookies and biscuits require post-treatment of heating or baking, which may alter its shape. Aregawi et al. were able to

3D print cookies of desired shape. They observed that changes in thickness of the printed cookies changed the Young's modulus of cookies. The structure of the printed cookies was dependent upon the printing technique and the ratio of flour: binder composition was significant in determining the structure of cookies (Aregawi et al., 2015). Vancauwenberghe et al. printed different structures of 3D-printed cookies and observed that the texture of the printed cookies depends upon the mechanical properties of the matrix material, such as the moisture content and the microporosity of the material. These properties in the matrix greatly effects the texture as the E modulus may change after the baking process (Vancauwenberghe et al., 2017a, 2017b).

The baking process greatly alters the structure and texture of the 3D-printed products as the components used for printing, such as carbohydrate, fat, protein, and moisture content are sensitive to heat treatment. A major challenge in 3D food printing is compatability of the printed food material with the traditional food processing technologies, such as baking in the case of cookies. Lipton et al. observed that the shape stability reduced by the amount of butter/fat in "cookie mixture" whereas an increase in egg yolk concentration increased the width/length stability but decreased height stability (Lipton et al., 2010). The high amount of fat makes it difficult to maintain its shape after postprocessing (in this case, baking). The deformation in final product due to this treatment deteoriates the quality of the printed product. Thus, the feed ingredients need to be modified with addition of certain binders that can help to maintain the shape stability. Kim et al. observed that addition of hydrocolloids such as xanthum gum can help retain the structure of cookies even after baking. Addition of xanthum gum 0.5 g/100 g were able to exhibit a texture profile similar to those of control cookies (Kim et al., 2019).

Pizza is another traditional food consumed in many parts of the world. 3D printing of pizza also requires some postprocessing treatment and is subject to shape stability. Thus, recipe modification is required to maintain the 3D model after heat treatment also. NASA's funding to SMRC (Systems and Materials Research Consultancy) of Austin has demonstrated 3D printing of pizza by extrusion method as a part of their contract. The dough for printing required extra water and thickeners in the tomato sauce to enable the printability of pizza. For cheese toppings, cheese was melted prior to the extrusion and laid over the sauce surface. This method reduced the timing of printing pizza (30 minutes) as compared to molding (24 hours—including fermentation time) (Lipton et al., 2015).

Limitations

Although a number of food ingredients have been 3D-printed till date, however, there still exists a research gap in 3D printing of food. The major challenge is the retention of the shape of the product after printing and postprocessing treatment. Compared to the traditional cooking, food ingredients have to be altered for their rheology, stability, and binding ability. For example, cookies tend to not retain their shape due to high content of fat (Lille et al., 2018). In these cases, such as cookies,

pasta, and pizza, posttreatment of the printed products includes heat treatment such as frying, baking, or steaming. These treatments may alter the texture of the printed food. Research is required to maintain the shape and texture even after the heat treatment to these 3D-printed products.

There exists a lot of food materials that are not printable in nature, such as rice, vegetables, and fruits. They have a lot of water content and specific food chemistry between the ingredients. A solution to this problem is the use of hydrocolloids in the solid matrix to enable a mouthfeel of these materials. Cohen et al. (2009) were able to produce different levels of mouthfeel (stiffness vs granularity) ranging between liquid to solid vegetables from different combinations of xanthium gum and gelatin only (Cohen et al., 2009). They chose two orthogonal axes (1) weak to firm, and (2) smooth to granular texture in comparison to food material. 0.5% gelatin resulted in milk like texture while 4% gelatin resulted in mushroom like texture. A combination of hydrocolloids were used to obtain different granularity—1% gelatin:4% xanthan resulted in risotto like texture while 1% gelatin:8% xanthan resulted in tomato like texture. An increase in xanthan:gelatin ratio resulted in increase in granularity.

Another major issue is the cost of printing and the time taken to print the final product. In the current scenario, a 3D printer is costlier than the traditional cooking methods. The time is also a major limitation in exploring the full potential of 3D printing—for example, baking a single cookie may take upto an hour. 3D printing may not be applicable to mass production of food products (Lin, 2015).

Conclusion

3D printing or additive manufacturing is finding new dimensions in the area of biotechnology. 3D printing in food sector is relatively new technology. Customized food designs and flavors and robotics-based cooking have been practised before the introduction of 3D printing. Researchers are exploring new ways to implement this new and exciting technology for different food materials and complex geometrical designs. 3D printing provides the flexibility for novel food designing and alternate methods of cooking and ingredient use. However, despite the recent studies, there still exists a research gap that needs to be addressed to fully explore this technology. The major challenge is the printing of nonnative printable materials, such as fruits, vegetables, and meat. Even for printable materials such as cheese and chocolate the process should be optimized before the commercial use. Further research is required understand the effect of 3D printing on both micro- and macrostructural and textural properties of the printed model.

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Three-dimensional printing for waste management

7

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Introduction

With the increasing rate of production of finished goods and products, the rate of waste generation is also increasing. Environmental conditions have become detrimental with increasing problems of pollution, resource depletion, biodiversity loss, and excessive land use. A transition to a more sustainable solution is the need of the hour. To address the financial and economic instabilities, the concept of Circular Economy has emerged. Although there are many definitions of Circular Economy, Geissdoerfer et al. defined it as “a regenerative system in which resource input and waste, emission, and energy leakage are minimized by slowing, closing, and narrowing material and energy loops. This can be achieved through long-lasting design, maintenance, repair, reuse, remanufacturing, refurbishing, and recycling” (Geissdoerfer, Savaget, Bocken, & Hultink, 2017).

The Circular Economy lists five main areas that need immediate attention for waste reduction—plastic waste, waste generated in construction sites and buildings, food waste, raw materials during production of finished goods, and bioproducts or biomedical waste. Recycling is considered to be the best strategy for waste reduction and for production of new materials (Mwanza & Mbohwa, 2017). Three-dimensional (3D) printing is considered to be a promising technique in recycling waste materials into new consumer-driven products. It can serve as a clean processing technology that can address the problem of waste generation and have a significant contribution toward it (Pinho, Amaro, & Piedade, 2020). In the present chapter, we discuss about how 3D printing is helpful in different areas of waste management and how it can be used as a tool for bioremediation process.

Three-dimensional printing for plastic waste treatment

Plastic-based goods have been increasing exponentially in the recent years. Most of the materials used in the production of plastics are nonbiodegradable and account for major environmental pollution. Plastic waste is generally managed in three ways—re-extrusion, mechanical recycling, or the use of thermal or chemical methods. Currently, only a small percentage of the plastics is recycled and nearly 80% ends up in a landfill (Mikula et al., 2021). However, land filling is not a solution, and there is need to look for other new solutions for plastic waste management. There has been an effort to recycle plastic waste; however, low economic benefit, variations in different forms, and limited number of times it can be recycled are factors that hinder the recycling process.

There exists a huge gap between production of plastic material and its recycling. A cost-effective method is the need of the hour to address the increasing production of plastic waste. 3D printing is a potential solution for the reuse of plastic materials. 3D printing allows for circular production systems that can recycle and produce finished goods from the waste material (Despeisse et al., 2017).

The most popular being the fused-deposition modeling (FDM). Most of the reported literature have used FDM technology for the production of products from recycled plastics. A commercial 3D printer RepRap has been used for the extrusion of filament from waste plastic wherein the recycled filament has been used to produce 3D prints (Baechler, DeVuono, & Pearce, 2013).

FDM is also generally associated with poor surface finishing and low mechanical strength. Although there are several challenges that need to be addressed before it can be employed for a closed loop style (Richardson & Haylock, 2012). Cunico et al. used FDM for 3D printing of plastic waste through a chemical recycling process. They observed dimensional distortion of around 7.9%; however, the mechanical strength improved for more than 20 times as compared to untreated objects. The process as proposed by the researchers reduced the plastic waste by an average of 9% during the process. Thus, more research is needed regarding the sustainability of the technology (Cunico, Kai, Cavaleiro, & de Carvalho, 2019). In recent times, ocean plastic has also become a major concern. In a study by Vones et al., 3D-printing was used, i.e., single screw extrusion, for plastics recovered from ocean waste. The prototype developed by the authors was used to create awareness about sustainability among school-going children (Vones, Allan, Lambert, & Vettese, 2018). Gaikwad et al. utilized e-waste plastic samples for 3D printing of finished goods. The 3D-printed objects had 76% and 83% of breaking and tensile strength, respectively, compared to the virgin plastic counterparts. The filament production process produced 28% less CO₂ as compared to the finished products from virgin plastics (Gaikwad et al., 2018). Although there are more existing literature on plastic waste management through 3D printing, we only discussed a few examples as it is out of the scope of this book.

Three-dimensional printing for food waste

According to a European Commission report in 2014, food waste is classified into three categories: (i) loss during food processing or production phase, (ii) loss during consumption phase—this loss is generally unavoidable and includes organic matter such as fruit peels and shells, (iii) loss during consumption phase—which could be avoidable and includes parts that were not eaten or is spoilt (Commission, 2014). Food waste management is a greater issue in developing countries than developed countries, mainly due to inefficiency in waste segregation. The food waste when not segregated from the solid waste leads to increased greenhouse gas emissions (Thi, Kumar, & Lin, 2015). The generated food waste is generally incinerated or is dumped in an open area. In both the cases, there is a great amount of greenhouse gas generation. Incineration also usually results in generation of dioxins, a major environmental concern. 3D printing can be an alternate solution to food waste management problem (Thi et al., 2015).

Recently, a number of papers have been published that use 3D printing for food waste management. Cocoa shells, a waste generated from cocoa processing industry, can be used as a biocompatible ink for 3D bioprinting applications. Tran et al. combined cocoa shells with poly(ϵ -caprolactone) polymer for bioink fabrication. The printed structure showed good adhesive property between layers and smooth resolution. The printing parameters did not have any significant change in the crystalline structure and the thermal and mechanical properties were sufficient to be used as a bioink (Tran et al., 2017).

Three-dimensional printing for wastewater treatment

Several parts of wastewater treatment process have been designed using 3D printing technology, which will be discussed in detail. The material or the polymer required for such purposes should have high mechanical strength, superwettability, and should not generate any toxic compounds. However, 3D-printed products usually have poor tensile strength due to lack of interlayer bonding. Recent research is focussed on increasing the strength of the membrane as well as the wettability (Yusoff et al., 2022). 3D printing technology enables freeform designing, less use of solvents, and low wastage.

Three-dimensional printing of filter membranes

Porous membranes are the first choice in water filtration. The membrane filters out the suspended solids allowing the freshwater to pass through. There has been extensive research to improve the selectivity and permeability. With 3D printing, hierarchical structures and scale can be precisely designed and fabricated. Due to resolution and material limitations, 3D printing is not yet widely used for developing direct polymeric membranes. The majority of 3D printers available today

cannot print below submicron resolution, where membrane pores are usually located. In addition, some membrane applications require specific types of material characteristics and wettability (hydrophilic or hydrophobic), which is challenging since 3D printers can only print certain materials. There are still some research groups that are demonstrating that 3D printing can be used for membrane fabrication, usually in the form of composite membranes, wherein the substrate is 3D-printed and the active layer is fabricated using other techniques (Tijing et al., 2020).

Lv et al. fabricated porous membranes for oil-water separation by 3D printing polydimethylsiloxane (PDMS) ink with nanosilica. A uniform PDMS membrane was produced by printing parallel PDMS filaments on the PFTS-treated glass substrate and heating for 1 h at 120°C followed by peeling off the substrate. In contrast to traditional methods, 3D printing provided a superhydrophobic surface on a porous framework, preventing weak interface adhesion. This had a separation efficiency of up to 99.6% (Lv et al., 2017). In another study, a selective laser-sintering method was used to fabricate a superhydrophobic polysulfone membrane support. The final 3D-printed membrane was made using a single polysulfones (PSU) powder layer sintered together. The performance of the membrane was tested after it was sprayed with candle soot. Besides exhibiting superhydrophobicity, the membrane also demonstrated chemical stability when exposed to acidic, basic, and neutral solutions. Even after 10 cycles, it displayed very high separation efficiency of over 99% for all hexane/water separations (Armbruster, Brochard, Lölsberg, Yüce, & Wessling, 2019).

Shimerry et al. 3D-printed composite membrane by depositing polyethersulfone on ABS-like support layer (with both flat and wavy structures) using multijet printing. The polyethersulfone was casted upon the support layer by phase inversion method. The authors checked for permeance, rejection, and cleanability by passing oil-in-water emulsion. The wavy structure was reported to have better efficiency than the flat structure. It had 30% higher permeance and 52% higher permeance recovery ratio than the flat structure. The 3D-printed wavy composite membrane was able to maintain some level of permeation even after five cycles of filtration, whereas the flat structure was fouled after the first cycle itself and cleaning with NaOCl of the wavy structure restored about 70% of the original permeance (Al-Shimmery, Mazinani, Ji, Chew, & Mattia, 2019). Another study by the same group observed that the wavy structure can overcome the issue of fouling more efficiently than the flat structure. The wavy structure was able to retain 87% of the initial pure water permeance even after 10 filtration cycles. This efficiency is due to the localized fluid turbulence induced by 3D-printed wavy structure. Fig. 7.1 demonstrates fabrication, the membrane, and the porous nature of the membrane by Scanning Electron Microscope (SEM) images (Mazinani, Al-Shimmery, Chew, & Mattia, 2019). Though initial results are promising, the report lacked information on the potential delamination of the selective layer for long-term operation, and the challenge of upscaling or modulation as the 3D-printed support layer may be too stiff and not easy to bend for module preparation. Further studies can be done to overcome these shortcomings.

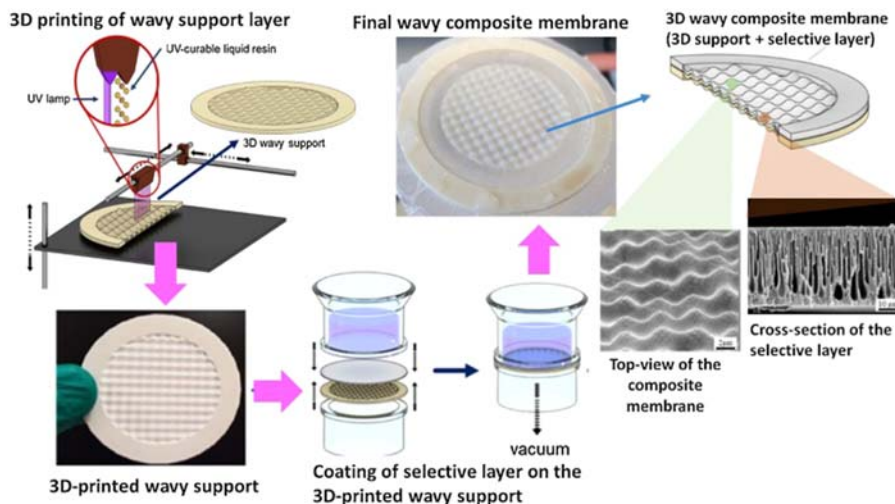


Figure 7.1 Fabrication of the 3D printed wavy design, the selective top layer was casted by nonsolvent induced phase separation and then the casted membrane was attached on the 3D printed support by vacuum filtration.

Source: Reproduced with permission from Tijing, L. D., Dizon, J. R. C., Ibrahim, I., Nisay, A. R. N., Shon, H. K., & Advincula, R. C. (2020). 3D printing for membrane separation, desalination and water treatment. *Applied Materials Today*, 18, 100486., Al-Shimmerly, A., Mazinani, S., Ji, J., Chew, Y. M. J., & Mattia, D. (2019). 3D printed composite membranes with enhanced anti-fouling behaviour. *Journal of Membrane Science*, 574, 76–85. and Mazinani, S., Al-Shimmerly, A., Chew, Y. M. J., & Mattia, D. (2019). 3D printed fouling-resistant composite membranes. *ACS Applied Materials & Interfaces*, 11(29), 26373–26383.

Three-dimensional printing of channel spacers

Channel spacers play an important role in designing membrane filters as they ensure fluid mixing, recirculation, and continuous flow. They are designed in various shapes and sizes with a goal to enhance turbulence, reduce fouling, and maintain mechanical support to prevent damage to the active layer. Spacers are usually made of polypropylene material and need to have a good balance of stiffness and flexibility as the dead zones may create space for particle deposition, ultimately leading to fouling and reduced mass transfer rate. The material should also have good chemical resistance as it supports the active layer. Since spacer can be of different shapes, the conventional techniques of manufacturing can be difficult and time-consuming. Thus, 3D printing can be helpful in the designing of complicated geometries (Lee et al., 2016; Tan, Chua, Chong, Fane, & Jia, 2016).

Thomas et al. fabricated a triply periodic minimal surface spacer designed to improve flux performance and increase fouling resistance. As compared to a commercial feed spacer, the design showed 60% improved flux performance and 63% overall film heat transfer coefficient (Thomas et al., 2018). In another study, an electrically polarized graphene-poly(lactic acid) spacer was developed using 3D printing followed by

electrical polarization in a high voltage electric field as depicted in Fig. 7.2. Its performance was evaluated in terms of water flux, reverse solute flux, and ion attraction compared to a 3D-printed nonpolarized graphene-poly(lactic acid) spacer. The electrically polarized spacer increased local osmotic pressure across the membrane surface, which increased the water flux. It also decreased the gypsum scaling on the membrane due to dispersion effect of electrostatic forces between gypsum aggregation and negatively charged surfaces (Yanar, Yang, Park, Son, & Choi, 2021).

Altering the spacer design also influences the energy consumption of the membrane filtration. Ali et al. developed 3D-printed column type feed spacer to reduce the energy consumption. By reducing the diameter of the spacer filament, the proposed spacer increases clearance between the filament and membrane without compromising flow channel thickness. The higher clearance reduces the flow unsteadiness, so columns type nodes were added to the spacer structure to shade the vortex. The 3D computational fluid dynamics (CFD) simulation of fluid flow in the channel of this spacer was compared to that of the standard spacer. Based on the numerical results, the proposed spacer reduced the pressure drop, shear stress, and dead zone substantially. An experimental investigation of the filtration performance of these spacers in a lab-scale filtration module confirmed these findings experimentally. In addition to reducing pressure drop by three times, the column spacer also doubled the specific water flux (Ali et al., 2019). Among the materials used for 3D printing of spacers—acrylonitrile butadiene styrene, polypropylene, and natural poly(lactic acid)—diamond-shaped spacers were fabricated. All had better performances in terms of mechanical strength, water flux, reverse solute flux, and fouling than commercial spacers and poly(lactic acid) that exhibited better performance than the other two due smooth surface, smaller exposed hole area, and better fouling resistance (Yanar et al., 2018).

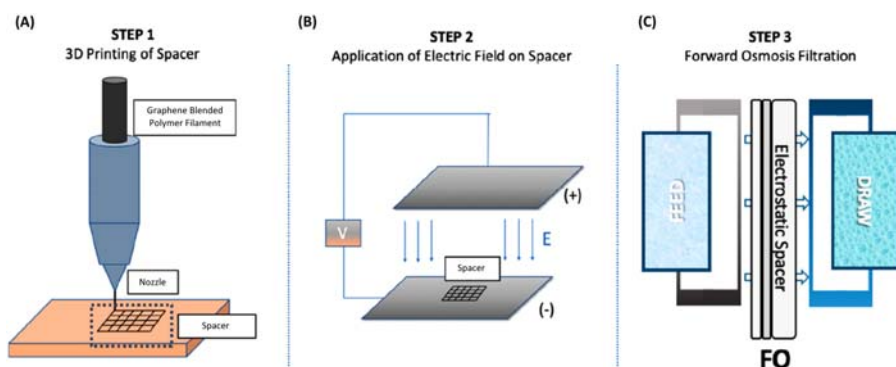


Figure 7.2 Fabrication and application processes of the electrostatic graphene-blended PLA spacers (A) 3D printing, (B) electric polarization, and (C) forward osmosis filtration.

Source: Reproduced with permission from Yanar, N., Yang, E., Park, H., Son, M., & Choi, H. (2021). Efficacy of electrically-polarized 3D printed graphene-blended spacers on the flux enhancement and scaling resistance of water filtration membranes. *ACS Sustainable Chemistry & Engineering*, 9(19), 6623–6631.

Three-dimensional printing of capsules and bio-carriers

Recently, 3D printing is being applied to manufacture 3D-printed bio-carriers. It is being done to enhance the performance of bio-carriers using complex geometries that can shelter the microbes and also promote the mass transfer. 3D printing of synthetic biofilms has a number of advantages as depicted in Fig. 7.3. The surface area and the topology of the design is very important in deciding the efficiency and performance of the bio-carrier (Elliott et al., 2017). 3D printing of gyroids is relatively new which is interconnected with minimal straight lines. A gyroid is a

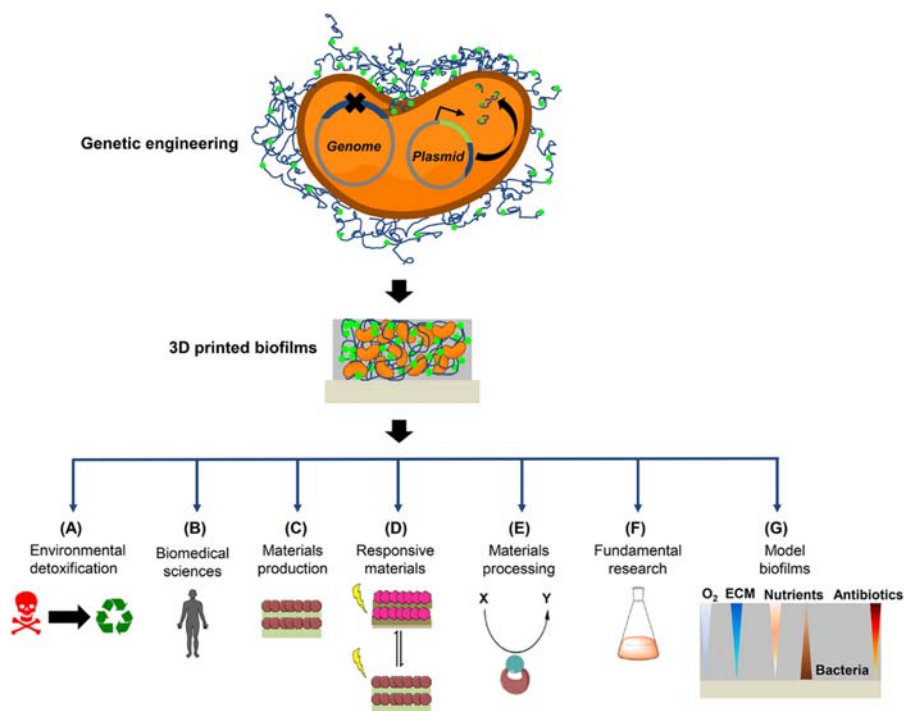


Figure 7.3 Possible applications of 3D-printed synthetic biofilms. Bacteria can be genetically engineered to produce structural biofilm proteins (in blue) decorated with specific functional peptides (in green) via heterologous expression in a bacterial strain that has a genetic deletion for structural biofilm proteins. By combining these engineered bacteria with 3D bioprinting, 3D-printed engineered biofilms can be created with multiple potential applications, including (A) Environmental detoxification and bioremediation, (B) Biomedical applications, (C) Tunable materials production with improved mechanical and/or conductive properties, (D) Fabrication of responsive materials, (E) Biocatalysis-driven materials processing, (F) Addressing fundamental research questions, and (G) Creation of reproducible model biofilm systems for studying the structure–function relationships of bacterial biofilms. *Source:* Reproduced with permission from Balasubramanian, S., Aubin-Tam, M.-E., & Meyer, A. S. (2019). 3D printing for the fabrication of biofilm-based functional living materials. *ACS Synthetic Biology*, 8, pp. 1564–1567. ACS Publications.

structure with high number of channels and interconnectivity to obtain the desired surface area. These channels are important to enhance the nutrition uptake and number of microbial colonies. A high surface area and channeled structure of gyro-id bio-carriers may induce anerobic conditions at specific locations, which promote the growth of nitrifying bacteria. However, these channels are also prone to clogging due to biofilm deposition. As a result, the overall efficiency decreases due to the hinderance in mass transfer of oxygen and nutrition.

In a study to investigate the effect of semisuspended bio-carriers on dissolved oxygen profiles and mass transfer in moving bed biofilm reactors, Tang et al. used 3D-printed semisuspended bio-carriers. Semisuspended bio-carriers combine the benefits of fixed and suspended bio-packaging, avoiding clogging and biomass loss caused by collisions. Polyhydric alcohol and isocyanate are used to fabricate semisuspended bio-carriers. Stereolithography was used for fabrication. A spindle-like shape was produced, and the smaller end of the carriers was attached to the frame (Tang, Zhao, Bin, Huang, & Fu, 2017).

Using fused deposition modeling technology, Wu et al. reported a multichannel wavy filler. Its performance was compared with that of a hollow polyhedral ball. Carriers were tested under the same conditions in different bioreactors (Wu, Xie, Zhao, Qi, & others, 2020). Numerical analysis indicates that the multichannel wavy filler provides a static hydrodynamic environment for microbes. Compared to polyhedral hollow fillers, this allows for faster biofilm formation. This occurs when the fluid's velocity decreases as it flows through the filter channels. By increasing vorticity, the multichannel wavy filler promoted oxygen and nutrients mass transfer. Additionally, the multichannel wavy filler increases the gas face distribution and traps bubbles, increasing the gas retention in the reactor, making dissolved oxygen more available to the biofilm. As a result, dissolved oxygen (DO) improves process efficiency.

Czolderova et al. used 3D printing to produce polyvinyl alcohol (PVA) capsules for ferrate capsulation, and the capsules were used in an industrial wastewater treatment process. 3D-printed capsules maintained a higher ferrate stability of 61% after 30 days of storage, whereas ferrates that were not encapsulated and those that were encapsulated commercially decomposed completely after only 14 days. It may be due to the high reactivity of ferrate toward moisture on capsule walls (Czölderová et al., 2018).

Three-dimensional printing for bioremediation

3D printing has enormous scope in the area of bioremediation. 3D printing is being employed in various applications such as detection of toxic chemicals, oil spill filtration, or biocatalytic living materials. Previously, 3D printing had only been used for metals or plastics, however, it can be also be used for the printing of living structures. In a recent study by Schaffner et al., they were able to print live bacteria into functional complex structures. The functional living ink was termed as "Flink" where bacteria was encapsulated in a hydrogel bioink consisting of hyaluronic acid (HA),

κ -carrageenan, and fumed silica. These materials of desired shape were able to degrade pollutants and also was able to produce cellulose (Schaffner, Rühls, Coulter, Kilcher, & Studart, 2017). Flink loaded with *Pseudomonas putida* was capable of phenol degradation while *Acetobacter xylinum* was capable of producing cellulose when exposed to oxygen in culture media. In another study by Balasubramanian et al., they were able to 3D print genetically engineered *Escherichia coli* biofilms. They used alginate bioink mixture and were able to cast on calcium containing agar surfaces, thus, a hydrogel can be casted of complex geometry with encapsulated bacteria. The printed structures were able to tolerate harsh chemical treatments and also remained viable (Balasubramanian, Aubin-Tam, & Meyer, 2019).

Bioremediation is more advantageous than the traditional methods of chemical oxidation or physiochemical adsorption as it is more cost-effective and does not cause any secondary pollution. This was demonstrated by He et al., wherein they 3D printed a bacteria-microalgae coculture. The “dynamic bioink” as termed by the authors is a microbe-laden hydrogel that was able to maintain metabolic activities in fermentation and bioremediation process. The *Bacillus*–*Chlorella* coculture system was used for the removal of acrylamide and methyl orange. *Bacillus subtilis* was used to convert these chemicals into CO₂ by using it as a carbon source and then *Chlorella vulgaris* can convert the released carbon dioxide into oxygen by photosynthesis (He et al., 2022).

Another application is for oil-water separation where 3D printing has been applied for superhydrophobic membranes. Lv et al. fabricated superhydrophobic membranes using hydrophobic nanosilica-filled PDMS ink. The printed structure was porous in nature with improved mechanical strength. 3D printing can overcome the weak interface adhesion issue that generally arises from the traditional method of coating on a mesh and it was also possible to control the pore size of the membrane. The authors were able to attain an efficiency of approximately 99.6% with a pore size of 0.37 mm (Lv et al., 2017). 3D printing can also be used to form membranes that can form superhydrophobic structures. Yang et al. 3D-printed micro-scale artificial hairs with an eggbeater head. The structure was inspired by *Salvinia molesta* leaf and they used immersed surface accumulation 3D printing process. The study revealed that a hydrophilic material can behave as a hydrophobic material if the microstructure is altered. To enhance the surface roughness and improve the mechanical strength, multiwalled carbon nanotubes were added to photocurable resins. The authors were also able to control adhesive forces (ranging from 23 to 55 μ N) by altering the number of eggbeater arms. Thus, 3D-printed eggbeater structure can be used in oil/water separation application (Yang et al., 2018). Although a new technology, 3D printing proves to have better efficiency and explores new possibilities of printing a structure that can alter the properties at micro level and have different biotechnological applications.

Conclusion

Recently, 3D printing is looked upon as a promising and convincing innovation that can have positive impact on waste management. It has received significant attention

as it provides opportunities to print new designs that are more sustainable and robust. Various advantage of 3D printing, such as the flexibility in material selection, altering the properties of the material due to design parameters, minimizing waste production, helped overcome the drawbacks of conventional manufacturing. 3D printing can now used to address the major challenge of plastic and food waste. Although there are some interesting literatures, it is still in nascent stage and needs to be improved before it is available at a commercial level. 3D-printed food from waste still needs consumer acceptance. There has been a major development in the use of 3D printing in waste water treatment with innovation in design of membranes and geometries. These membranes can be scaled up for wastewater treatment at a larger scale. 3D printing in bioremediation is still challenging and requires improvement in sustaining the viability of biocatalytic living materials. The key challenges need to be further investigated and optimized to fully understand and realize its potential in terms of accuracy, cost, and scalability.

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Emerging trends of three-dimensional printing in biotechnology

8

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Introduction

In spite of rapid progress in the previous decade, three-dimensional (3D) bioprinting has also faced many challenges related to biology, medicine, biomaterials, and biotechnology. Recent trends and technologies are required to precede the technology of bioprinting in various aspects. Therefore the current chapter deals with emerging trends of 3D bioprinting technology with new possibilities.

Recently, 3D bioprinting is performed using multimaterial based ink. When 3D bioprinting is done using a single material, the physicochemical properties of printed objects are limited. To address this problem, there is growing interest in modifying and diversifying generic printing materials by combining other materials with distinct properties. For example, combining a bioink formulation with a polymeric scaffold or fillers or additives like nanoparticles and nanoplatelets with distinct properties to generate 3D printable composites. These printed composites show high efficiency, optimal mechanical properties, and desired biocompatibility and are helpful in manufacturing living tissue or whole organs. However, for precision in printing whole organs, development of computational model and related software is crucial. Furthermore, computational tools such as machine learning, etc., are used to assess the complexity and accuracy of fabricated bioparts. Tissue-engineered scaffolds, for example, are typically very complex because they promote

cell growth in a predictable manner to achieve corresponding functions. If precision can be analyzed in advance using machine learning, the final manufactured bio-parts can be assured to be of high quality. This chapter discusses about multimaterial 3D printing, computational models, machine learning as a computational tool and software used in bioprinting.

Often, in situ bioprinting is also done, which refers to the direct creation or repair of living tissues or organs at their original site. However, few limitations such as need for remarkably effective printable bioink with the capability of rapid solidification in a living person and need for a biologically acceptable ink for enhanced tissue formation limits the use of in situ bioprinting. Furthermore, regulatory issues related to living models are also available, which need safe and sterile tissue delivery. Since in situ bioprinting holds great promise in operating rooms in the near future, the possibilities and methods for in situ bioprinting of various tissues and organs along with the strategies for translating the technology from bench to bedside are critically discussed in the chapter.

It is now possible to identify not just qualitative but also quantitative quantities of numerous genes, transcripts, and proteins at the same time using omics technology. The chapter includes the recent trend in bioprinting, that is, the use of multi-omics and direct gene printing that helps in minimizing the errors in manual handling of 3D bioprinter and failures in the bioprinted products. Moreover, use of bioprinting in the production of vaccines, medicines, and delivery systems, as well as the present vaccine platforms and treatments used in the treatment of numerous infectious illnesses, are also focused upon.

In the concluding sections, the chapter focuses on 4D and 5D bioprinting. The 4D bioprinting combines the fourth dimension, that is, time with 3D bioprinting technology. Objects can be produced quickly due to the recent bioink materials' rapid fusion, folding, and remodeling capabilities, allowing bioprinting in the fourth dimension, "time." This will be a game-changing technology that, in the short term, will allow mass fabrication of living tissues for pharmaceuticals and provide an alternative strategy for potential organ-printing technologies. The 5D bioprinting provides print head and the printable objects with five degrees of freedom. It accurately creates curved layer or concave shapes according to design requirements. The printed part moves as the printer head prints in five directions at the same time. The print bed moves forward and backward together with the x , y , and z axes, allowing the item to be printed from all five axes instead of only one.

Emerging trends of three-dimensional bioprinting in biotechnology

Multimaterial three-dimensional bioprinting

3D bioprinting with a single material usually do not produce objects having diverse physicochemical properties thus; restrict their use for wide medical applications.

Therefore in recent past there is a growing interest in modifying and diversifying generic printing materials by combining diverse materials with distinctive properties, such as combining the bioink with a polymeric scaffold or by incorporating fillers and/or additives with unique properties. These fillers and additives may include nanoparticles, nanoplatelets, and fibers, etc., to generate 3D printable composites with superior efficiency, desirable mechanical, and biocompatible properties (Ashammakhi et al., 2019; Le Duigou, Castro, Bevan, & Martin, 2016; Lee et al., 2014; Zhang et al., 2016). This section deals with the most recent advancements in 3D multimaterial printing along with diverse printable composite or hybrid materials, as well as their prospective applications in tissue engineering and other biomedical fields.

Printing technologies using multimaterial

Modern multimaterial 3D printers may build items with diverse and gradient functionality using a multihead printer that fabricates composite materials that are combined sequentially or simultaneously with precise control of material composition and properties.

Fig. 8.1A–C shows multilayered mixed composite scaffolds mainly composed of single plastic support ink and nonplastic bioink. The scaffolds that support inks are generally prepared from polycaprolactone (PCL), polylactic acid (PLA), or copolymers of these materials, which are printed using fused deposition modeling or normal extrusion and heated to the glass transition or melting temperature. This plastic is the main component of 3D printable composites, as it maintains the composite materials' structural integrity and ensures consistency during the printing process (Ashammakhi et al., 2019). It functions as a mold around the bioink to keep it from overflowing in one layer (Fig. 8.1A and B), or as a stiff distinct separate layer between two consecutive bioink layers (Fig. 8.1C). Mixing of PCL or PLA layers with alginate, agarose, gelatin, or Matrigel bioinks has been widely proven to be helpful as bone and/or cartilage transplants (Daly, Critchley, Rencsok, & Kelly, 2016; Müller, Öztürk, Arlov, Gatenholm, & Zenobi-Wong, 2017; Shim et al., 2016a). They are also used in the creation of durable cell culture platforms (Verma Atul, 2018). Shim et al., for example, successfully used a multihead deposition system to print a multilayered and cell-rich cyto-compatible composite material using PCL and chondrocyte cell-encapsulated alginate hydrogel in different layers, resulting in outstanding cartilage reconstruction in mic (Shim et al., 2016a). The process of cell-encapsulated alginate hydrogels printing did not affect the vitality of chondrocytes. The technology permitted scaffold creation while also depositing adjustable numbers of cells and growth factors in pretissue constructions with precise spatial location. After 4 weeks, histochemical analysis of the retrieved implants demonstrated increased cartilage tissue and type II collagen fibril production in the printed PCL-alginate hybrid scaffold with no adverse tissue reaction. This approach of 3D printing multilayered constructions could have many uses in tissue engineering.

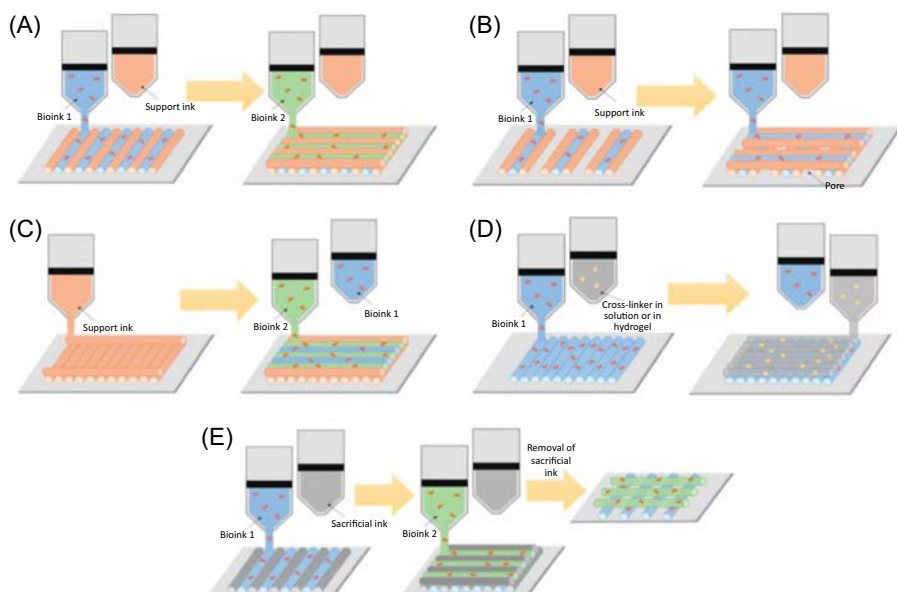


Figure 8.1 Printing of composite scaffolds through multihead printer containing a plastic support ink (A–C) and two nonplastic (bio)inks (D and E) (Mao et al. 2020).

Source: Reproduced with permission from Mao, H., Yang, L., Zhu, H., Wu, L., Ji, P., Yang, J., & Gu, Z. (2020). Recent advances and challenges in materials for 3D bioprinting. *Progress in Natural Science: Materials International*, 30(5), 618–634. <https://doi.org/10.1016/j.pnsc.2020.09.015>.

Another method of producing desirable characteristics of composites with multi-layered sequential alternating structure, incorporating at least two different nonplastic bioinks, is heterogeneous layer-by-layer printing (Fig. 8.1D and E). Specific cells or network precursors, such as cross-linkers, photoinitiators, or ions, can be encapsulated by each bioink. Li et al., for example, described for the first time a reliable alginate/methylcellulose (Alg/MC) blend hydrogel for 3D bioprinting, along with the technique to increase adhesion between printed layers (Fig. 8.2) (Li, Tan, Leong, & Li, 2017). The optimized Alg/MC blend hydrogel was extremely thixotropic, with excellent extrudability and stackability. The interfacial bonding between the printed layers was greatly improved after treatment with a trisodium citrate (TSC) solution. The TSC solution served as a chelating agent, removing the calcium ions from the surface of each layer. After 3D printing, post-crosslinking in a CaCl_2 bath improved the adhesive strength between the layers even further. With the help of TSC, the Alg/MC hydrogel displayed enhanced printability, stackability (150 layers could be printed), and shape fidelity. A freshly 3D-bioprinted Alg/MC construct had a good cell viability of >95%.

In heterogeneous layered printing, any printing material can be used as a temporary sacrificial template. Postprinting, this material can be easily removed without

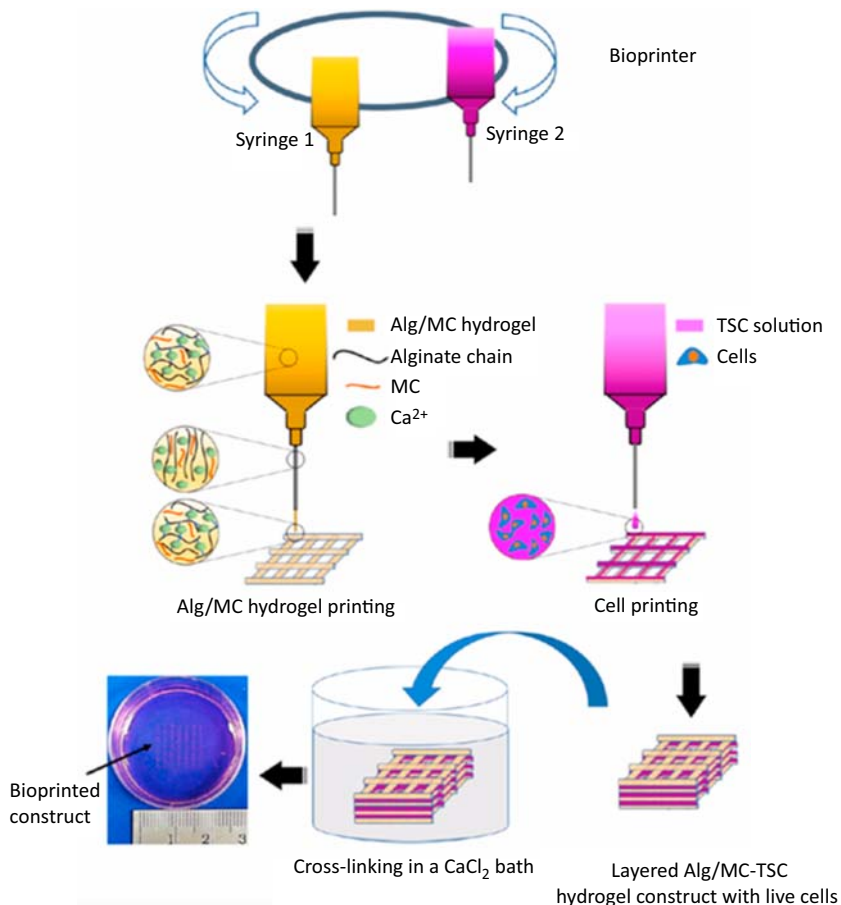


Figure 8.2 Schematic illustration of the extrusion-based bioprinting process with the Alg/MC hydrogel and cells-TSC solution. The construct is built layer-by-layer, wherein each layer is formed by extruding the Alg/MC hydrogel from syringe 1, followed by extruding a cells-TSC solution from syringe 2. The construct is postcrosslinked in a CaCl₂ solution before culturing at 37°C in a cell culture media.

Source: Reprinted (adapted) with permission from Li, H., Tan, Y. J., Leong, K. F., & Li, L. (2017). 3D bioprinting of highly thixotropic alginate/methylcellulose hydrogel with strong interface bonding. *ACS Applied Materials and Interfaces*, 9(23), 20086–20097. <https://doi.org/10.1021/acsami.7b04216>. Copyright (2017) American Chemical Society.

sacrificing the printed structure (Fig. 8.1E). In an attempt to print bioblood-vessel (BBV) through coaxial cell printing technique, the pluronic (CPF-127) was extruded inside, whereas a hybrid bioink composed of vascular-tissue derived dECM along with alginate was extruded outside (Gao et al., 2017). This composite material provided a desirable environment for cellular proliferation, differentiation, and neovascularization, and hence, allowed tubular BBVs to be directly

manufactured. Furthermore, by adjusting the printing conditions, the BBVs can be made in a variety of sizes.

While multimaterial 3D printing integrates diverse material properties into one printed object and gives essential stability and functionality for functional ones, the interfacial performance between different materials with entirely different properties may restrict the printed composites' structural reliability. Reactive groups bound on polymer chains that allow effective bonding between numerous material interfaces consequently resolve the compatibility issues. Wirthl et al. created a group of adhesives able to connect hydrogels and hostile materials instantly and stably (Wirthl et al., 2017). Tough hydrogels firmly adhere to plastics, leather material, bone, and even metals in seconds, achieving exceptional interfacial toughness of more than 2000 J/m².

Hybrid/composite materials for three-dimensional bioprinting

As previously stated, switchable and flexible nozzles or core–shell/coaxial printing are widely used to combine different materials into one object with precise distribution in multiple layers or zones or regions, enhancing the functionality of the printed object. Individual printer head is filled with one substance in this situation, and the printing ingredients are separated before printing. Another method of multimaterial printing is to introduce suitable doping agents into generic printable materials and/or preblend other bioactive components. This gives the resulting printed composites varied physicochemical and biological properties. The difference between the two techniques in multimaterial printing lies in the use of single-head printer for combining materials and isolate pure materials prior to printing in one method, while in other in the use of multihead printer to combine and hybridize materials before printing.

Doping nano-objects into generic printed materials can result in hybrid structures. Precise additives, like nanoparticles and fibers have been used to provide the composite material adequate toughness and improved mechanical strength (Chimene et al., 2018; Tan, Tan, Yeong, & Tor, 2016). Gaharwar et al. introduced a nanoengineered ionic-covalent entanglement (NICE) bioink formulation to manufacture the sophisticated cell-laden 3D composites with mechanical rigidity, high elasticity, and improved printability, as illustrated in Fig. 8.3. Nanosilicates are used to enhance an ionic covalent entanglement hydrogel developed from bioactive GelMA and kappa-carrageenan (CA) in the NICE bioinks. The NICE bioink is able to print self-supporting, high aspect-ratio structures over 3 cm and 150 layers tall with good shape conformity, and after cross-linking, the structures become strong and elastic, capable of supporting more than 50 times their own weight. Throughout the 120-day timeframe, cells embedded the 3D-printed object maintained superior viability and proliferated, which is critical for long-term cell or tissue regeneration. NICE bioinks are used to bioprint sophisticated, cell-laden constructions with great structural conformity and mechanical rigidity for tissue engineering applications.

Apart from the nanoobjects listed above, it is worth noting that adding relevant chemicals or bioactive compounds to the printed objects might also adjust their

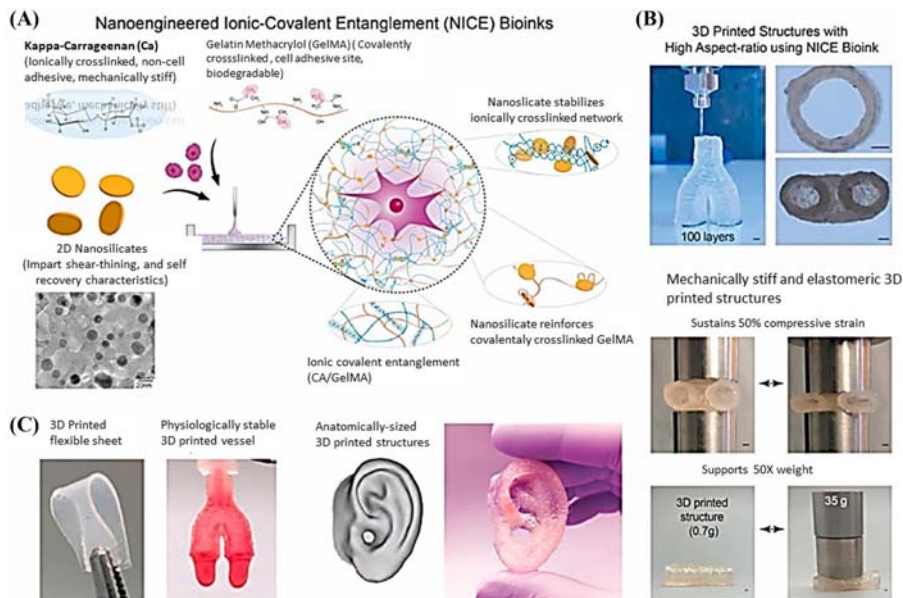


Figure 8.3 Tough, elastic, and highly printable NICE bioinks. (A) The NICE bioinks use nanosilicates to reinforce an ionic—covalent entanglement hydrogel made from GelMA and κ CA, creating a dually reinforced hydrogel network. (B) The NICE bioinks print mechanical stiff and elastomeric hydrogel structures with a high aspect-ratios and high print fidelity (scale bar = 1 mm). (C) The 3D-printed structures from NICE bioink are mechanically (film) and physiologically (bifurcated vessel) stable and have high structural fidelity (3D-printed ear).

Source: Reprinted (adapted) with permission from Chimene, D., Peak, C. W., Gentry, J. L., Carrow, J. K., Cross, L. M., Mondragon, E., ... Gaharwar, A. K. (2018). Nanoengineered ionic-covalent entanglement (NICE) bioinks for 3D bioprinting. *ACS Applied Materials and Interfaces*, 10(12), 9957–9968. <https://doi.org/10.1021/acsami.7b19808>. Copyright (2018) American Chemical Society.

properties (Huang, Zhang, Gao, Yonezawa, & Cui, 2017). The composite can be easily manufactured in these circumstances by physically combining the modifiers compounds with bioinks. For example, the growth factors can be included with the matrix material to obtain cellular differentiation and improve the bioink's biological properties (Jang et al., 2017). The angiogenic cytokine vascular endothelial growth factor (VEGF) is involved in the proliferation and differentiation of endothelial cells. VEGF encapsulated in heart tissue generated dECM bioinks was found to serve a significant function in generating strong vascularization and tissue matrix development in vivo in studies (Fig. 8.4) (Chimene et al., 2018). The patterned patch also improved cardiac functions after myocardial infarction, including reduced cardiac remodeling and fibrosis, as well as cardiomyogenesis and neovascularization.

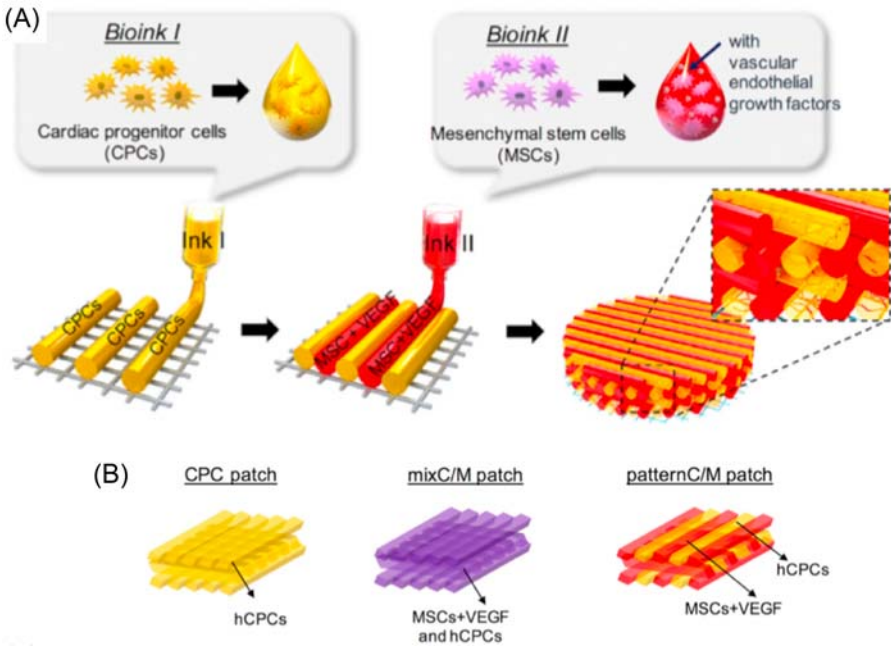


Figure 8.4 Stem cell-laden dECM bioinks encapsulated with VEGF for 3D bioprinting of complex tissue construct. (A) Illustration of prevascularized stem cell patch including multiple cell-laden bioinks and supporting PCL polymer. (B) Schematics of the experimental groups (Mao et al. 2020).

Source: Reproduced with permission from Mao, H., Yang, L., Zhu, H., Wu, L., Ji, P., Yang, J., & Gu, Z. (2020). Recent advances and challenges in materials for 3D bioprinting. *Progress in Natural Science: Materials International*, 30(5), 618–634. <https://doi.org/10.1016/j.pnsc.2020.09.015>.

Softwares used for bioprinting

In bioprinting technology, the computational model is essential. It is the first problem that must be addressed when bioprinting is used to create living tissue or an entire organ. Consequently, software development continues to lag behind bioprinting progress (Gulyas, Csiszer, Mehes, & Czirok, 2018).

Softwares for controlling printer

The majority of currently available software is for controlling the bioprinting process. These include graphical user interfaces-based control tools, such as GeSim's Bioscaffolder. This program works with STL or stereolithography files that depict the surface of an item. Scaffold-generator and STL-interface are the two modules. The Organovo firm produced general software for its NovoGen MMX Bioprinter that only contains core operations. It comes with a graphical user interface for creating various 3D structures. It enables a user to select parameters such as materials,

different types of cells, and speed of printing process as well as writes and loads predefined commands for executing certain robot motions and deposition heads. Organovo is now joining hands with Autodesk to develop bioprinter control software ([RegenHU, 2020](#)). For their 3D-Bioplotter system, EnvisionTEC has created computer-aided design and computer-aided manufacturing (CAD-CAM) software with a user-friendly graphical interface. This software was created to keep track of and regulate the printing process till end.

To control the bioprinting process, CELLINK created their specific software suite called HeartOS, DNA Cloud, and DNA Studio. It is for quick droplet layered printing, and the model may be loaded in G-code or STL format. This software package is simple and easy to use and does not require prior learning. The user may easily alter parameters like flow and speed with the software. The software also allows the user to get a preview of model prior to printing, run a slicing preview to reveal the effect of individual layer setting on final outcome, and use dynamic tools for infill density ([Cellink, 2016](#)).

Allevi created the AlleviBioPrint Pro bioprinting control software ([Impression 3D et Imprimante 3D _ Meilleur Prix, Comparatif, News, n.d.](#)). It is web-based, allowing users to access it from any computer. AlleviBioPrint Pro offers integrated slicing, project-based workflow, and built-in model development. Furthermore, the software has radical visualization properties and an interface that allows users to visualize and amend possible issues in their projects prior to printing.

In situ bioprinting

3D-bioprinting provides the concept of predesigning the constructs to specifically fit into the defect/wound site. Usually, these constructs are fabricated ex vivo and then implanted at the defect/wound site ([Tellisi, Ashammakhi, Billi, & Kaarela, 2018](#)). However, this mechanism has many disadvantages associated with arrangements, modifications, sterility, trimming, adjustments, or to combine several pieces to fit size and shape of treated defect/wound site. Moreover, the change in the shape and size of the fabricated construct may not always fit into the debrided defect/wound site. To avoid these challenges the concept of in situ 3D bioprinting was introduced. Therefore in situ bioprinting, which is also known as in vivo bioprinting, refers to direct bioink printing at the defect/wound site to repair or to create living organs or tissues ([Guillemot et al., 2010](#)) with the help of either the handheld printers ([Di Bella et al., 2018](#); [Duchi et al., 2017](#); [Hakimi et al., 2018](#)) or robotic arms assisted with printer nozzles. These are managed through scanners and/or computers that regularly measure the exact size, shape, and place of defect/wound ([Wang et al., 2015](#)). Upon full installation, the technique of in situ bioprinting can produce precise and accurate refabricated tissue defects and can result into more efficient and faster healing of the defected tissues. However, in this novel approach, some of the printing devices have been reported that utilized the application of in situ bioprinting ([Binder et al., 2010](#); [Di Bella et al., 2018](#); [Keriquel et al., 2010, 2017](#); [Sofokleous, Stride, Bonfield, & Edirisinghe, 2013](#)) ([Fig. 8.5](#)).

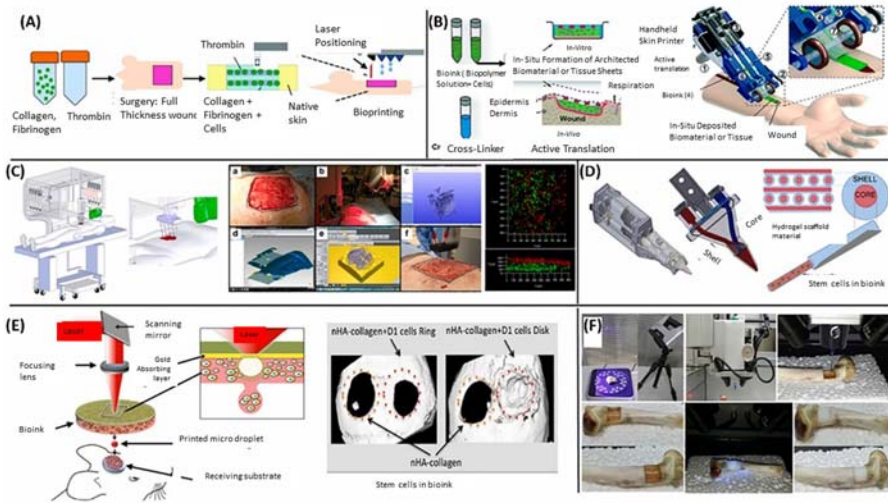


Figure 8.5 Current status on in situ bioprinting approaches: (A) Robotic, pressure-driven inkjet bioprinter-assisted printing on mice, and amniotic fluid-derived stem (AFS) cell uniformity in the 24-h postprinting harvested gel. (B) Schematic diagram illustrating the working principle of a handheld in situ skin bioprinter. (C) System design and working concept of the robotic, motor-driven inkjet bioprinter for large animals. (D) Core-shell bioprinting with a co-axial handheld in situ bioprinter (Biopen) for cartilage tissue application. (E) Schematic representation of the laser-assisted bioprinting (LAB) approach and advantages of printing in disk geometry. (F) In the process of 3D bioprinting to fill the bone defect, a 3D scanner was used to obtain accurate images for generating the CAD model, which was followed by printing and photopolymerization by exposing UV light (Singh et al., 2020).

Source: Reproduced with permission from Singh, S., Choudhury, D., Yu, F., Mironov, V., & Naing, M. W. (2020). In situ bioprinting – Bioprinting from benchside to bedside? *Acta Biomaterialia*, 101(xxxx), 14–25. <https://doi.org/10.1016/j.actbio.2019.08.045>.

Bioprinting can be done using various techniques, such as droplet-based bioprinting, extrusion-based bioprinting, laser-assisted bioprinting, electrohydrodynamic bioprinting, and core shell deposition. The techniques used in in situ bioprinting should rely on the development of targeted organ with satisfactory resolution and cell viability (Datta et al., 2018). For in situ bioprinting, time is also considered as one of the most important parameters because low and poor printing speed bioprinters makes the process difficult to accomplish in the clinical settings.

Machine learning in three-dimensional bioprinting

An application of artificial intelligence (AI), machine learning (ML) offers the ability to inevitably and automatically learn and recover from experience without being explicitly trained and programmed. AI technique based on ML has direct impact on the quality of the final 3D-printed product, minimizes costs, and reduces fabrication

time. They help in the optimization of 3D printing by facilitating the growth of the printable biomaterials and also improve the performance and productivity of the available systems. There are certain other aspects related to the manufacturing process and the efficiency of the design for 3D bioprinting techniques, such as service evaluation, printability checking, nozzle path planning, etc. (Goh, Sing, & Yeong, 2020).

The HML technique was applied by Bone et al. (2020) to utilize a small dataset for developing high-reliability bioprinting predictions with alginate ingredients. The model used physical connections to develop links to print score parameters, which allow better precision for training as well as test data. LASSO has been used for model coefficient tuning as well as function selection of dominant physical causal forces driving the print error. Such optimization helped in predicting optimal construct parameters required for high-stability in prints, and an adjustment between the simplest printing components. This method was used through selecting the optimal content, process variables for a selected type to direct printing constructs. Known physical equations help extend our technique's potential generalizability to include a variety of inks that may include biopolymers, cellular products, and cells, which can be used to direct the printing of intricate structures with structural conformity and functional potential that are included in the figures of merit (Bone et al., 2020).

Utilizing the model system with naturally produced biomaterials, Lee et al. (2020) established an ML-based approach to build bioinks for 3D-printing. In this study, as compared to native collagen (NC), atelocollagen (AC) has essential physical properties for 3D printing. AC gel showed alterable behavior that was slightly elastic and temperature-sensitive, producing a soft creamy structure with lesser yield stress, while NC gel revealed irreversible behavior that was strongly cross-linked and temperature-sensitive that resulted in fragility and higher yield stress. Furthermore, it was identified that a general relationship is sustained by ML between the mechanical properties of bioink and its printability. ML supported a high elastic module that improves shape conformity and feasible extrusion at lower degree of yield stress that was found critical. Furthermore, different formulations of naturally derived bioinks based on this relationship have been derived that provided high degree of shape conformity through multiple-regression analysis. Lastly, a 3D object composed of cell embedded hydrogel with a bioink structure of high shape conformity was fabricated that advocated about the 3D constructs, high cellular viability, and proliferative properties (Lee et al., 2020).

Bioprinting based on stereolithography (SLA) can generate complex 3D structures accurately and efficiently. However, in this SLA-based method, ultraviolet (UV) irradiation poses major challenge that can kill cells. Due to the intricate cellular structures and cellular activities, models based on concepts of physics are not capable of precisely predicting the viability of cells. Utilizing ML approach, Xu et al. (2020) established a predictive model for cellular viability. Keeping the effects of important process parameters, such as UV strength and exposure time, gelatin methacrylate concentration and thickness of layers, several experiments were performed to obtain experimental data under various bioprinting conditions. To predict cell viability under different bioprinting conditions, a collaborative

learning algorithm having a combination of neural networks, ridge regression, K-nearest neighbors, and random forest (RF) was established. The functional performance of this computational model was calculated on the basis of three error metrics. Furthermore, RF was used to assess the value of each process variable for cellular viability. It has been shown that model can efficiently predict cellular viability with high accuracy and also evaluate the influence of each process variable in SLA-based 3D bioprinting on cellular viability (Xu et al., 2020).

Ruberu et al. (2020) introduced ML as a new method for quantitatively testing printability and rapidly optimizing extrusion printing to achieve a reproducible 3D scaffold. To optimize 3D bioplotting through a scoring system setup to evaluate the printable property of gelatinmethacryloyl (GelMA) and hyaluronic acid methacrylate (HAMA)-based bioinks, Bayesian optimization has been used as an ML method. The efficiency of two basic parameters across the printing process has been integrated into the scoring metric along with filament forming and layer deposition in 3D scaffold. The optimal print parameters for GelMA containing inks with varying concentrations [10%, 7.5%, and 5% (w/v)] were observed in 19, 4, and 47 experiments, whereas 32, 25, and 32 experiments were needed for GelMA:HAMA [10:2%, 7.5:2%, and 5:2% (w/v)]. In contrast to the 6000 to 10,000 possible combinations in the Bayesian algorithm, the number of experiments was significantly reduced. This research will therefore be a step toward unraveling the advantages of ML in this rapidly evolving field of 3D bioprinting (Ruberu et al., 2021).

Direct gene printing

In 3D bioprinting, the relation of molecular structure to its function is highly crucial and the scarcity of this knowledge leads the way to various errors in the handling of 3D bioprinting devices and finally the printed biological structures failed to perform its function. Depending on the development of dynamic, 3D-printed models, information on their biochemistry also needs to be considered while developing such machines. Likewise, the molecular information, interactions, functions, and specific mechanisms provided by nucleic acids can lead to the development of better delivery systems. Currently, methods that are used for DNA synthesis show several limitations, ranging from the oligonucleotides lengths, generation of errors in the correct genome sequencing process, time consumption, low yield, and toxic chemical reagents used in the reactions, failure to adhere to DNA sequences and use of toxic solvents, and laboratory environmental conditions. Direct gene printing (DGP) is one of the areas that are gaining importance, as it focuses on providing a pathway for efficient nucleic acid production and also to eliminate the limitations faced earlier in their production. For advanced medications and other healthcare applications, synthesis of DNA sequences may be essential. Cambrian Generics, a San Francisco-based biotechnology company, used a laser-based technique to print DNA. On metal beads, the DNA is conjugated, scanned, and assessed by a computer system to locate the correct synthesized sequences. A computer-assisted laser beam is then bombarded with the correct DNA sequence, which is further collected on a glass plate, while the influence of this beam causes the beads to display the

correct sequences. In the processing of billions of strands at once, this method can be extremely useful. To further establish ideal or exceptional gene sequences, it could be used to enhance or change the functions of the body, for example, by engineering the microbial genome for the creation of new drugs or by modifying the protein sequence in the cancer treatment (Stein, 2015; 3ders, 2019; 3D Printing Industry, 2014). In the future, the applications can be further modified for gene editing, wherein the defective gene can be efficiently sorted and eliminated or synthesized/replaced by the edited (modified) functional gene. For cloning (into a plasmid vector) and benchtop synthesis of linear DNA fragments with an efficiency rate of approximately ~83%, Synthetic Genomics developed an automated 3D bioprinter known as BioXp™ 3200 system. This system produces DNA clones just in overnight incubation from personalized reservoirs of oligonucleotides prepared from the required sequence data and then inserted into the cloning production process. In this, the gene sequence to be cloned is first submitted (as input data) to the software, after this step, the custom solvents are chosen and the system is loaded, then the DNA fragments are sorted and cloned. It performs manual functions automatically, such as thermal cycling, mixing, pipetting, purification, and storage (Alvarez, 2015; DNA Printer for Gene Production. NOVO Engineering, n.d.). Gene editing approaches, such as the CRISPR/Cas9 technique, are a bacterial immune system modification using a single guide (sg) RNA to further activate the Cas9 endonuclease at the site of action (Martinez-Lage, Torres-Ruiz, & Rodriguez-Perales, 2017). This further leads to DNA cleavage at the targeted site. Further developments in technologies such as CRISPR/Cas9 technique widely applicable in genome engineering and its integration into 3D printing applications may emerge as more potent gene therapy processes (Martinez-Lage et al., 2017). Similarly, Tesla, Inc. is said to have built mobile molecule mRNA printers or “RNA microfactories” to develop mRNA-based COVID-19 vaccines (Reuters, 2020).

Multomics studies of three-dimensional bioprinted constructs

Individual omic studies, such as epigenomics, genomics, proteomics, transcriptomics, and metagenomics, aimed at understanding a specific problem in human disease, agriculture, plant science microbiology, and the environment have been successful to a great extent in the past decade. More broadly, implementing multiple omics research frequently results in datasets with a wide range of data modalities derived from several assay types, as well as increased dimensionality (Krassowski, Das, Sahu, & Misra, 2020).

Omics technology enables massively parallel examination of substances, such as genes, transcripts, and proteins. This technology has the goal of generating a large number of data in just a single experiment and within a limited period it had an unmatched throughput. With this advancement, it is now possible to identify not just qualitative characteristics but also quantitative features of numerous genes, transcripts, and proteins at the same time using omics technology. Furthermore, because of the technology's increased sensitivity, specificity, and accuracy, critical

biomarkers for illness prediction can be identified considerably more quickly (Kim et al., 2017).

Antitumor therapeutic screening in vitro models currently available has several critical constraints. Several chemicals are reported to inhibit cell growth in 2D culture and have different pharmacological effects in humans, wasting both human efforts, material resources, as well as time taken for drug development. As a result, new models must be developed. When compared to sandwich culture and organoid creation, 3D bioprinting technology offers more advantages in the synthesis of human tissue. A 3D model was created with HepG2 cells using 3D bioprinting technology (3DP-HepG2). Immunofluorescence, real-time quantitative 3DP-HepG2 demonstrated considerably enhanced expression of tumor-related genes, such as AFP, ALB, CD133, CD24, IL-8, EpCAM, and b-TGF as compared to standard 2D-cultured tumor cells (2D-HepG2). Transcriptome sequencing revealed significant gene expression differences between 3DP-HepG2 and 2D-HepG2, particularly in the genes that are linked to hepatocyte functioning and malignancy (Sun et al., 2020).

The microenvironment has a big influence on how cells behave. Traditional 2D cultures of liver cells generally lack the hierarchy and structural components of the organ, and as a result, the stability of the developed model is found to be poor (Bale et al., 2014). In comparison to 2D monolayer cultivated primary human hepatocytes (PHH) in extended culture periods, 3D-cultured human hepatocytes preserve their periportal and perivenous phenotypes and display higher activity of drug-metabolizing enzymes and transporter proteins due to increased heterotypic cell-cell interactions. The 3D (co-)cultured liver microtissues derived from PHH have achieved long-term functional, phenotypic, and metabolic stability. It also remained susceptible to the exposure of any chronic hepatotoxin during the culture period, according to the transcriptome, proteomic, and metabolomic investigations (up to 5 weeks) (Bell et al., 2016).

To study the drug-induced hepatotoxicity under normal and inflammatory conditions, few research studies have been performed using omics techniques. Jiang, Pieterman, Ertaylan, Peeters, and de Kok (2019) study the mechanisms of acute (24 hours) and chronic (3–14 days) conditions using PHH/Kupffer cells (KC) co-cultured 3D liver microtissues. They studied the liver spheroids having PHH and measurable levels of KC and stellate cells through oleic and palmitic acid concentration to better understand the pathophysiology of fatty liver diseases. The most elevated lipid classes following the lipid injections were determined using high-resolution mass spectroscopy analysis of the long-term (generally considered as 21 days) metabolomic responses to the disruptions (Jiang et al., 2019).

Lung biofabrication is a recently developed technique for tissue engineering and regeneration that involves seeding the cells into scaffold (an acellular organ) and cultivating these cell-loaded scaffold in a bioreactor to find new therapeutic targets (Farré, Otero, Almendros, & Navajas, 2018). In the drug discovery process, biofabrication is applied. Proper disease modeling is required for the development of newer and better treatments. COVID-19 vaccines are being developed by several research groups. To find therapeutic targets for COVID-19-induced lung infection,

2D/3D lung models are being developed. Due to complex biological changes under natural environment, 2D humans and animals cell models cannot meet the study's requirements (Alsafadi et al., 2017; Yadav et al., 2021).

Engineering vaccines and therapeutics using three-dimensional bioprinting

Vaccines and therapeutics development (antibiotics including antiviral medicines, antibodies, and other therapeutics) typically takes more than 10 years of time and nearly 3 billion dollars on average to cover the complete process, that is, from research to market entry. Recently, nano- and microtechnologies have been used for the manufacture of therapeutic agents, including vaccines to accelerate the drug development process and increase the efficiency of drug delivery and efficacy of new medicines to solve major pharmaceutical challenges. This section discusses the prospect of 3D bioprinting technology adopted for the production of vaccines, medicines, and delivery systems, as well as the present vaccine platforms and treatments used in the treatment of numerous infectious illnesses.

3D bioprinting technology, which is already being used in tissue engineering, is quickly becoming a strong approach in pharmaceutical manufacture and medication delivery. Personalized medicine, which needs extensive production procedures due to customized dosages and precise drug combinations, can now be manufactured using 3D printing technology. The 3D bioprinting technologies for directly generating RNA vaccines and medicines are described in this section.

RNA printer (CureVac)

To combat any epidemic conditions, a proof-of-concept has been built for the mRNA printing facility for quick formulation and distribution of vaccines. The aim is that the RNA printer would be able to produce a clinic-ready vaccine after designing and validating the number of RNA vaccines in parallel with the identification and/or sequencing of the causing new pathogen. CureVac is one of the pioneers of mRNA printing technologies and develops mRNA vaccines against a variety of viral illnesses, including yellow fever, Lassa fever, MERS, and COVID-19 (Armbruster, Jasny, & Petsch, 2019). Its first goal was to create a reliable, automated, or semiautomated system for delivering the company's rabies vaccination.

Drug printer

The pharmaceutical industry adopted 3D printing to generate high patient-specific dosages based on structural designs. The controlled release qualities of the 3D-printed tablets reduced the frequency, that is, the number of dosages per day consumed by a patient in their therapy period. Reduced dose frequency boosted patient compliance and extended the release of products for individuals who took the medicine multiple times a day (Wheless & Phelps, 2018). That is, instead of taking a medication every few hours or days, a patient can take one dose in the morning.

Spritam is a reformulated version of the antiepileptic drug levetiracetam developed by Aprelia Pharmaceuticals (Agrawal & Gupta, 2019). ZipDose, a unique powder bed and inkjet 3D printing technology, was used to develop the first 3D-printed fast release tablet that disintegrates in a few seconds when dissolved in an aqueous solution. The inkjet printing head drips a binding liquid over a powdered layer, similar to selective laser sintering (SLS). Layers can be layered up to 40 times depending on the size of the tablet, allowing for more compact medicine packaging. This medicine was approved by the FDA in 2015, and a single tablet earlier carrying 200 mg can now contain 1000 mg (Agrawal & Gupta, 2019). The resulting tablet is a high-dosage drug that is easy to ingest, breakdown when consumed, and provides a consistent dose over a certain period in persons with epilepsy.

Fina, Goyanes, Gaisford, and Basit (2017) used SLS technology with Kollicoat IR or Eudragit L100–55 to help the sintering process by utilizing a Candurin gold shine to make a paracetamol tablet. The printed tablet, known as a printlet, has good mechanical qualities, such as low friability (0.02%–0.56%) and strong crushing strength of about 284–485 N, and had a theoretical value of medicines. The pH-independent release characteristic of the Kollicoat-based formulation was observed, with a release rate dependent on the drug content, whereas the pH-dependent release profile of the Eudragit-based formulation was not connected with drug loading.

Khaled, Burley, Alexander, Yang, & Roberts (2015) used 3D extrusion-based printing technique to create a polypill to treat diabetic patients with hypertension (Khaled, Burley, Alexander, & Roberts, 2014). The polypill is made up of a connecting layer, a zero-order drug-based captopril osmotic pump compartment, and nifedipine and glipizide sustained release compartments. When the patient takes the pill, soon the connecting layer of the pills breaks down, separating the polypill into captopril and a sustained-release chamber. By adjusting the porosity for diffusion, 3D printing technology allows manipulation of two distinct release profiles: first the sustained release of ramipril, atenolol, and pravastatin, and the second the quick release of aspirin and hydrochlorothiazide. Patients with several risk factors, such as hypertension and dyslipidemia or any other combination of diseases, can be treated with advanced medication by prescribing only a single tablet that has this ideal release profile.

Some people, on the other hand, require many medications with more complicated release profiles. According to Sun and Soh (Sun & Soh, 2015), 3D-printed tablets customized according to the need can achieve the required release profile. The tablet is made up of three parts: (1) a medication that contains a surface-eroding polymer, (2) a drug-free surface-eroding polymer, and (3) an impermeable polymer that acts as a protective layer. Surface-eroding polymers with drug compartments could be designed in various forms to achieve constant, rising, decreasing, and pulse release. These experiments show that 3D printing technology has huge potential in developing desired polypills in a single tablet as well as creating intricate and sophisticated medication with required release profiles. Although 3D printing integrated pharmaceutical production method has numerous limitations,

these approaches show promise as a cost-effective and efficient means to make customized tablets.

Four-dimensional bioprinting

4D printing is an advanced method of production based on 3D printing, where “time” is integrated as the fourth dimension. The 4D-printed constructs may change their shape, properties, or function with time under the influence of an external stimulus, such as temperature, water, pH, etc. Fused deposition modeling, microextrusion-based 3D printing, stereolithography, and digital light projection are some of the examples among the technologies that are being developed for 4D printing. Among the 4D printing technologies, microextrusion-based 3D printing due to its ease of use and wide range of ink materials, is one of the most widely accepted technologies (Sydney Galman et al., 2016).

4D printing is affected by five major factors: (1) the responsive material, (2) the additive manufacturing process, (3) the stimulus type, (4) the mechanism of interaction between stimulus and material, and (5) the mathematical modeling of transformation material (Tamay et al., 2019). The 4D printing technique can be linked to several prototypes printed by Tibbits of the Massachusetts Institute of Technology. For example, Ge et al. printed a strand that when immersed in water, auto-deforms from its original straight form into the letters “MIT” (Ge, Dunn, Qi, & Dunn, 2014). Later, 4D printing has modified into 4D bioprinting as it becomes compatible with biological materials or even living cells and was used to print into complex constructs. 4D printing cannot be referred to as 4D bioprinting until it meets any one of the following criteria: (1) Printed objects, such as biomedical devices, that can be used in biomedical engineering. (2) The printed construct is biocompatible so that it can be transplanted into the human body. (3) The printed materials are laden with living cells. The 4D printed bioconstruct will be able to modify its shape, size and/or functionality over a period of time (Gao et al., 2016; Li, Zhang, Akpek, Shin, & Khademhosseini, 2017). We all know that a mechanism of evolution often occurs spontaneously without any stimulus. However, in the presence of any external stimulus, mechanism of evolution is more stimulated. Printed objects may be converted into very complex structures that are difficult or even impossible to acquire through conventional methods or even through 3D bioprinting too. For example, hollow structures and other complex 3D structures are difficult to produce, such as blood vessels, as collapse as soon they are printed. Certain techniques (e.g., sacrificial material method) have been established to make the vasculature; however, the results are still not very satisfying. Another issue with 3D bioprinting is that it takes into consideration only the initial state of the printed biological structures and paid little attention to the complex revolution that generally occurs after printing. 4D bioprinting has emerged as a modern platform that enables a potential solution to overcome the limitations of conventional fabrication techniques. In addition, the bioconstructs can alter their functionality by 4D bioprinting.

Based on the nature of the mechanism of triggering, the stimulus used in 4D bioprinting, it is classified into: (1) physical stimulus (such as shear stress, acoustic light, temperature, light, electric and magnetic field), (2) chemical stimulus (such as gases, ions and pH), and (3) biological stimulus (enzymes) (Koetting, Peters, Steichen, & Peppas, 2015; Li, Guo, & Zhang, 2021). Temperature is the most common method for changing the behavior of a bioprinted material. At lower critical solution temperature or upper critical solution temperature, temperature-sensitive hydrogels such as poly(*N*-isopropylacrylamide), gelatin, and agarose undergo a reversible volume change due to the breakdown or expansion of the polymeric chains in the solvent (Altomare et al., 2018). Polyelectrolytes having carboxyl, phosphate, sulfonic, and pyridine as functional groups are sensitive to the pH of the surrounding medium and are commonly used in tissue engineering (Wan, Zhang, Liu, Lv, & Zhou, 2020). Under the reaction conditions, when the charges present in the functional groups are neutralized, the intramolecular and intermolecular forces that are regulated by the electrostatic interaction cause polymeric chains to undergo modifications in their structures, like from coil to globule (Dai, Ravi, & Tam, 2008).

Ion-responsive materials, such as Ca^{2+} and Zn^{2+} , where the crosslinking and dissociation of polymeric chains are based on ions, may use the same configurational transfer mechanism. Changing the type of the ions or their concentrations present in the reaction solution affects the degree of the electrostatic interactions, which controls the mechanical properties of the hydrogel. Due to this characteristic, ion-responsive hydrogels have been reported to modify the rheology of various bioinks before use in bioprinting, as well as after bioprinting. These hydrogels can modify the mechanical properties of the constructs to create a hierarchically ordered bone structure (Krishnakumar, Sampath, Muthusamy, & John, 2019). Due to the ability of alginate to react to metal ions like Ca^{2+} , it has been widely used in bioprinting (Virus, Disorders, Report, Author, & Author, 2012).

Lai et al. (2021) used the combination of alginate (Alg) and methylcellulose (MC) to develop a hydrogel having the property of being highly printable and shape-changing that can be used in 4D printing. The optimized Alg/MC hydrogel had excellent rheological properties, extrudability, and print shape fidelity. In 4D printing, the network density gradients were strategically regulated vertical to the direction of the patterned strips, as a result, Alg/MC hydrogel was printed into a series of patterned 2D architectures. This bioink provides anisotropic stiffness and swelling behaviors to the printed construct.

After dissolution in a calcium chloride solution, these 2D models could turn into various described 3D shapes simple (e.g., tube, curl, and helix) to complex (double helix and flowers) by manipulating the strip interspacing and angle. This shape-morphing Alg/MC hydrogel with outstanding printability has a greater possibility for 4D printing with delicate hydrogel patterns, which are becoming more important in regenerative medicine, diagnostic applications, and soft robotics (Lai et al., 2021).

Kim et al. (2020) define a 4D bioprinting system that is cell-friendly and biocompatible; including more than two digital light processing (DLP) and photocurable silk fibroin (Sil-MA) hydrogel forms of cells. After 3D printing, the shape changes of bilayered Sil-MA hydrogels have been controlled under physiological

conditions by modulating their internal or external properties. To explore the potential changes in the complex structure, we used finite element analysis simulations. Using this 4D bioprinting device, we eventually rendered mimetic trachea tissue with two cell types and implanted it into a damaged rabbit trachea for 8 weeks. The implants were incorporated naturally with the host trachea, and at the predicted sites, both epithelium and cartilage were created. These results show that the 4D bioprintingsystem can biologically generate tissue mimetic scaffolds and indicate the potential value for tissue engineering and clinical application of the 4D bioprinting system (Kim et al., 2020).

Five-dimensional bioprinting

Further advancements in the printing technique resulted in a new branch of additive manufacturing, that is, 5D printing, in which the print head and the printable object have five degrees of freedom. This technology is capable to generates curved layers instead of previous flat layers. In this method, during printing, the print part is kept in motion rather than the print head. As a result, rather than going through a straight layer as 3D printers do, printing follows the curve direction of the element being printed. The main benefit of this technology is that it allows you to make a component with a curved layer that is stronger. In 2016, American universities pioneered the principle of 5D printing. Mitsubishi Electric Research Laboratories is currently implementing it through their research team, including WilliamYerazunis as a senior principal research scientist (Zeijderveld, 2018).

5D printing technology uses a five-axis printing approach to create structures in different dimensions instead of the three axes that are used in 3D printing. Besides the X, Y, and Z-axis of 3D printing technology, the print bed is able to shift back and forth directions on two axes resulting in five-axis printing. 5D printing is capable of producing much stronger products as compared to 3D-printed parts (products). Ethera, an Indian company headquartered in Bengaluru, has been working on 3D printing technology for the past few years. They developed an idea to create a 5D printer, which they were able to do successfully and win the CES award for best invention.

A 5D-printed model can be used for artificial-bone therapy. Since bones have a curved surface, not flat, artificial bones made by 5D printing are designed to provide exceptional strength to these bones on replacement. This technology has a wide range of potential to meet this primary need. Since it uses flat layers, 3D printing is not very useful for making complex curved orthopedic implants. According to the analysis results, 5D-printed constructs are 3–5 times stronger than 3D-printed objects. It has also been determined that a 5D-printed cap is four times stronger than a 3D-printed cap and can withstand four times the pressure. During curved layers, there is a force of material that makes the printable object stronger. As a result, compared to 3D printing, less raw material is needed to make implants of the same strength (Ravinder Reddy & Anjani Devi, 2018).

The use of complex and powerful implants with curved surfaces is important in orthopedic surgery. 5D printing can print these complex surgical implants according to the patient's actual surgery and can also be used for surgical preparation, teaching, and learning. As a result, 5D printing can easily produce a complex and curved structure that needs a great deal of power (Gillaspie et al., 2016).

The primary distinction between 3D printing and 5D printing is that 5D printing produces a stronger component with a curved sheet, while 3D printing produces a flat surface. Both processes make use of the same technologies, such as 3D CAD file input, 3D printer, and 3D printing content. 4D printing, on the other hand, is not like these two innovations. It employs a variety of programmable materials that can change shape and work in response to time and temperature. Since they have thermomechanical properties, these materials are referred to as smart materials.

These smart materials can be used to manufacture implants for complex surgical cases using data from computer tomography (and magnetic resonance imaging scanning techniques in orthopedics). Different applications, such as 3D slicer, Mimics, OsiriX Imaging Software, Magics, 3D doctor, and InVesalius, can easily convert this data to 3D CAD file format for use in 5D printing (Haleem, Javaid, & Vaishya, 2018).

Conclusion

This chapter discusses the future of bioprinting science and technology in the coming decade. The emerging trends include multimaterial 3D bioprinting, software used for 3D bioprinting, in situ bioprinting to convert the technique into operating rooms and facilitate printing body parts on humans in the future; machine learning in 3D bioprinting to assess the accuracy of fabricated bioparts; direct gene printing for minimizing the errors in manual handling of 3D bioprinter and failures in the bioprinted products; multiomic technology for 3D bioprinting and engineering vaccines and therapeutics using 3D bioprinting. 4D bioprinting facilitates the tissue fusion and growth and development process for in vitro tissue fabrication processes; 5D printing has the potential to affect orthopedics in the future. It will open up a world of possibilities and provide outstanding care to save the patient's life. This technology appears to be capable of producing complex formed implants to meet urgent medical and orthopedic needs. While future trends show some promising ideas, there is still a lot of work to be done in many areas of bioprinting.

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Sustainability potential of 3D printing in biotechnology

9

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Introduction

3D printing (additive manufacturing, AM) techniques applicable to the field of biotechnology are also termed as 3D bioprinting. Recent technical advancements have enabled 3D bioprinting of biocompatible materials, cells, and supporting components into complex 3D functional living tissues with the aim of imitating natural tissue characteristics. With the foot forward into the era of digital revolution, there is an increased trend to revolutionize the commercial world through reassessing the ideas, rethinking and rebuilding the economic models, and reorganizing the processes to stabilize economic, environmental, and social needs, while leaving the same opportunities undisturbed for future generations to meet their needs. This chapter presents a discussion on a rapidly emerging technology, 3D bioprinting, as an extension of AM, which is now being considered a game changer in major sectors, including medical, bioengineering, and biotechnology and to completely reconfigure manufacturing and business ecosystems (Hsu & Jiang, 2019; Munaz et al., 2016; Zhang et al., 2019). The 3D printing or AM offers unique manufacturing procedure that allows successive layering to build a 3D object based on a digital model.

Initially, used for prototyping and modeling purposes, the gradual technological advances in process (printing technique), equipment (the printer), and materials used for printing (ink) have provided the application of 3D printing an upsurge in various industries including aeronautics, automobile, toys, and medicine and even food, to name a few. Gradually, emergence and inclusion of bioprinting in medical and biotechnology domain has offered popularity to this technique. The growth of

this market is attributed to technological advancements in 3D bioprinters and bio-materials, increasing use of 3D bioprinting in the pharmaceutical and cosmetics industries, and rising public and private funding to support bioprinting-related research activities. These factors have promoted market players to improve and strengthen their current manufacturing and distribution capabilities, especially in emerging markets, which are expected to witness the highest growth. Furthermore, the recent COVID pandemic is another major factor that has boosted various 3D bioprinting communities that are responding to the worldwide crises by offering their respective skills to ease the burden on the governments and supply chain of medical equipment as well as food.

As we strive for a sustainable future, industries are focusing on sustainable manufacturing and trying to optimize their manufacturing practices to decrease energy consumption and reduce waste production. 3D bioprinting is often observed as one among the key sustainable technologies, as it facilitates more efficient designs and less resource wastage. Despite being considered smart, sustainable manufacturing with less wasteful process, the questions remain open: where does 3D bioprinting stand on the green and sustainable scale? The topic has been surrounded with a lot of hype when predicting future applications, scope, and future revenue growth (Gebler, SchootUiterkamp, & Visser, 2014). It has even been discussed that 3D bioprinting will bring modern and developing societies to the doorstep of a new industrial revolution (The Economist, 2012). Moreover, effects of this technological innovation have hardly begun to be taken into account, and our intention is to study ongoing trends as well as to question what are the societal, environmental, and ethical implications of bioprinting trend.

Need for sustainability

In comparison to the traditional approach adopted by industries and organizations, recent times have seen sustainability as an important dimension to be considered when it comes to innovation and emerging technologies. This goes hand in hand with global awareness about the urgency to meet sustainability goals, both in the short and long terms. The sustainable technological advancement prevents negative environmental impacts, such as deterioration and contamination of natural resources, nourishes and replenishes supplements to nature reserves through production and conservation with efficient use of energy. The research and survey shows that sustainability is the main yet subtle aim of all organizational and technological innovations that give highest returns in terms of environmental and human benefits. Adopting a sustainable approach minimizing the cost, because companies end up decreasing the inputs they use. Additionally, sustainable processes produce better products that generate additional revenues and/or enables companies to expand businesses. Therefore, as these are the goals of corporate innovation, smart companies now treat and acquire sustainability as innovation's new frontier.

Environmental sustainability

Pertaining to the global environmental concern, there is an increased focus toward practices involving reuse, repair and refurbishment, remanufacturing, and recycling. The concept of manufacturing a product through adding raw (bio) material instead of subtracting it and producing waste gives a clear sense of environmental sensitivity and sustainability in bioprinting technique. In general, bioprinting involves the utilization of small amount of initiating material to produce the final product as in case of human tissue or organoid printing, wherein small number of stem cells is required to initiate the process or in case of 3D food printing, wherein small components are printed to assemble into final edible product. Thus, bioprinting is considered a resource-efficient technique that helps in reducing buy-to-fly ratio (the weight of raw material utilized in comparison to weight of final product), energy consumption, and carbon footprint (Peng, 2016).

Furthermore, discussing biomass waste management through bioprinting gives points to the technique for being environmentally sustainable. Biomass waste is produced from biological sources, such as living or dead livestock as well as relevant industrial by-products, such as cellulose and nanocellulose (Yu et al., 2021). The biomass waste is an important alternative energy source and also a unique source for various bioproducts used in many fields, including medical and biological research. Over the past two decades, a serious scientific attention has been given to reuse, recycle, and best recover various biomass wastes for high valued bioproducts. In bioprinting, domain cellulose, nanocellulose, and other biological wastes from plant, animal, and microbial sources have emerged as a catalog of high-valued renewable nanomaterials for variety of bioink formulations. These bioinks are used for diverse objectives in 3D bioprinting because of their structural similarity to extracellular matrices (ECM) and tremendous biocompatibility of subsidizing vital cellular activities (Lemahieu, Bras, Tiquet, Augier, & Dufresne, 2011; Wang, Wang, & Xu, 2020). Bioprinting offers the use of bioinks made from recycling of biomass waste at a significant low price. Over the past decade, many pioneering brand names have appeared that utilize biomass waste to produce bioink formulations for various purposes. Thus, bioprinting gives a practically suitable way to manage biomass waste.

In the food sector, bioprinting also promotes a multi-partite approach that includes diet variations, production of innovative edibles, and reduction in food waste. In this regard, the 3D food printing technique allows the use of waste from food industry, over ripen or rotten fruits and vegetables in bio(food)ink formulations, followed by blending and combining of various ingredients from residual food flows in the form of food ink to produce variety of edibles (Severini, Derossi, Ricci, Caporizzi, & Fiore, 2018). Furthermore, to mitigate the risk of ecosystem perturbation and climate change caused by livestock meat production, under the umbrella of bioprinting, 3D food printing has been recognized for its unprecedented potential to print edibles with complicated structures and low cost and energy efficiency (Lynch & Pierrehumbert, 2019). In a sustainable practice of printing meat or

its green substitutes, the possible materials are derived from in vitro animal and/or plant cell culture, meat by-products or waste, and plants and/or plant-based products (Soya, tofu etc.) (Dick, Bhandari, & Prakash, 2019; Ramachandraiah, 2021). Such food printing approaches are largely considered and discussed for their potential environmental sustainability.

Infact, bioprinting today bears the potential to be a green technology and also show the potential to be even greener with the inclusion of recycling techniques, raw material development advances, and low-energy printing of abundant, non-toxic, renewable, compostable, or biodegradable ingredients, with desirable physical and/or chemical properties. The sustainability of bioprinting technique could be improved by using common and/or renewable components, printer innovations (speed, precision, and productivity), optimization of fabrication logistics, and rational structure of supply chain and material flow. However, in current scenario, use of toxic, non-renewable materials with little consideration for resource conservation or environment safety is still continued, which is a serious issue that sidelines the sustainable potential of 3D bioprinting.

Economic sustainability

The recent burst of sustainability discussion on 3D bioprinting has revealed the technology as an ecofriendly, innovative method for creating 3D objects from renewable sources or through repurposing biodegradable or even non-degradable waste (Li, Chen, Fan, & Zhou, 2016; Sanchez-Rexach et al., 2021). In this way, bioprinting promotes re-entry of biological components safely into biosphere along with maintaining the circulation of technical components at high quality without entering or perturbing the biosphere (Unruh, 2018). Thus, 3D bioprinting fits well in the concept of circular economy that also seeks to make optimum use of resources to avoid waste (Unruh, 2018; vanWijk & van Wijk, 2015). In an economic system, circularity refers to the goal of eliminating or reusing waste and the persistent use of resources and in bioprinting domain it all depends upon the greater open-source collaboration between industry partners that could eventually provide new methods of addressing such sustainability goals with the technology (Stefenakis and Nikolaou 2021). Thus, bioprinting offers a major opportunity for companies to re-engineer their buy-make-move-fulfill supply chain for competitive advantage. Moreover, as 3D bioprinting emerged as a focused technique for precise, reproducible, and high-throughput production of intricate cell cultures and tissue constructs in drug testing and regenerative medicine, food products, dental, and other medical implants and biosensors, a fast growth of hardware manufacturers is observed in the market with different types of low-cost, technically advanced, and easy-to-handle bioprinter models. This has tremendously increased the market size of small, portable, and easy-to-use 3D bioprinters (Rahman et al., 2018; Zastrow, 2020; Wood 2021; Tong et al. 2021). These bioprinters have a wide range of underlying printing technology, possible add-ons/features making the selection process

rather daunting (especially for a non-expert customer). Furthermore, in combination with easy accessibility, online repositories of related softwares are encouraging the interest of users to produce personalized printed products. This may lead to a paradigm shift from consumers to prosumers (consumer that can produce) and influence the community on local scale. Thus, there could be four major ways through which 3D bioprinting can influence supply chains and drive the competitive advantages.

Decentralized production

Today's supply chain model is based on globalized economy. 3D printing enables onsite production, thus helps in shortening the supply chain leading to a low-cost product. It further allows direct production to local markets or customers. In the current state of corona pandemic, this is especially important as purchasing or collecting raw material from different locations has become difficult. Likewise, at the times of geopolitical tension, such as present Russia-Ukraine conflict or trade war, when the cost of purchasing components globally can increase suddenly, localized production is more helpful (Ben-Ner & Siemsen, 2017; Ihl & Piller, 2016).

Personalized product customization

Product customization ability gives an unprecedented freedom to design the product according to the specific and personalized requirements, for example, personalized therapeutics and medicines or personalized printed food (Fan et al., 2020; Lee, 2021; Zhenzhen and Yeng 2021). This will subsequently result in agile supply chains where designing, production, and distribution merge into one supply chain with a high potential of adapting changes in the market (Novak & Loy, 2020).

Less complexity and improved time-to-market

It is possible because of blending of various components and steps in one manufacturing process. This will lead to reduced complexities related to manufacturing, lesser production costs, enhancing lead times, and improving time-to-market.

Better resource efficiency, rationalized inventory, and logistics

3D printing provides better resource efficiency due to environmentally sustainable, energy and cost-efficient production method with almost negligible waste. The bioprinting technology utilizes the locally available raw materials to produce on-demand customized products, thus, lowering the risks related to overproduction, storage, and transportation of physical goods across countries and continents.

Another aspect that is quite considerable while discussing the economic sustainability potential of 3D bioprinting includes monetization of bioprinted product (Sun, Hua, Cheng, & Wang, 2020). The pricing strategies for bioprinted products or services seem problematic, because a range of products or services could be provided from a single platform at a time. However, the optimal pricing strategy for 3D-printed standard and

customized products include products' peculiarities with maximum profits to the producer and/or designer as well as maximum utility to the customer with the purchase of the final product in print. Moreover, in current scenario, the availability of biomaterials and technical advancements has accelerated the adoption of print-on-demand components that can be further assembled to develop final customized functional objects as in case of food printing (Guo et al. 2019). These printed components can also be highly personalized according to individual needs and requirements, for example, dental implants, skin implants, and food. At this point, although the companies provide customized services with basic design development by designers, but also allows the use of customers' personal data for designing the product. Thus, the final price of the bioprinted product depends upon designers intervention (basic design development), producers platform setup, and customization according to consumers requirements. Ongoing technological advancements in the existing 3D bioprinting technologies, ease in development of customized products, reducing costs of printers, digital manufacturing processes, availability of a range of biomaterials, easy manufacturing of custom-made products, government investments in bioprinting R&D projects, rising public and private funding to support bioprinting research activities, and advancements in industrial-grade 3D bioprinting platforms are some of the factors actively accelerating the growth of 3D bioprinting market. The 3D bioprinting market is projected to reach USD 1,647.4 million by 2024 from USD 651.6 million in 2019, at a CAGR of 20.4% from 2019 to 2024. This market size has been calculated on the basis of major bioprinting components, such as 3D bioprinters, bioink formulations (natural, synthetic, and blended mixtures), biomaterials (live cells or their components, hydrogels, and other natural or synthetic polymers), applications (medical, pharmaceutical, or drug research), and end user (pharmaceutical companies, individual customer, and academia). The market size of bioink (natural, synthetic, and blended mixtures) is predicted to reach US\$ 1.4 billion by 2024 from US\$ 306.2 million in 2019 at a CAGR of 35.4% over the period of 5 years (Gopinathan, 2018; Jhamb, 2019). This will certainly give a boost to economy of bioprinting sector. Furthermore, it is imperative to discuss that with the increasing market of bioprinted products, there is also an increased demand for the characterization of these products. This will certainly require basic to advanced biological science and interdisciplinary practices, such as multi-omics, single cell-omics, and genome editing. A common requirement of sophisticated equipment and reagents for these practices will also contribute to the market size of bioprinting.

Despite the localized and customized fabrication, inflow of bioprinted products in the market may also negatively influence the workforce requirements in different phases of traditional production and supply. Nevertheless, with the advent of this automated and computer-aided 3D bioprinting technology, development of new interdisciplinary areas, such as computational biology, Artificial Intelligence (AI) and Machine Learning (ML), Internet of Things (IoT), Augmented Reality and Virtual Reality (AR and VR), and engineering may inevitably lead to existing market expansion and greater job opportunities for new generation in technical work domains (Moreta-Martinez et al. 2020; An et al. 2021; Long Ng et al., 2020). Although the situation in this regard may not be linear due to disproportionate reality of skilled and non-skilled workers yet, in times to come, commercialization of 3D bioprinting technology will open new vistas in employment sector.

In addition, the bioprinting process directly relies on computational data and designing that too in majority of cases designed by experts of computational skills. In recent couple of years, there is an upsurge in the inclusion of ML, AI, AR, VR in bioprinting-related endeavors, for example, medical imaging, slicing and segmentation, optimization of bioink formulations, in vitro parametric studies, and bioprinting process that has attracted judicious scientific attention. Majority of these are the collection of computational approaches that are used to predict approximate mathematical functions of any complex mechanism or process depending upon its existing data. These computational approaches are inevitable and preferred in bioprinting because they tend to be more robust in the real-world context in terms of predication when compared to traditional mathematical simulations and models. Furthermore, while considering sustainable use of bioprinting technology in the future for tissue engineering and regenerative medicine, cost-effective robotic 3D bioprinting systems compatible with variety of bioinks are being developed (Kahin, 2019; Lan et al. 2021). Such custom bioprinting robotic arm allows multi-material printing that can fully synchronize with complexities of printing process and considerably reduce the overall set up cost (Lipskas et al. 2019; Tan et al. 2021). Automation and robots are known as viable means of increasing efficiency and cost- and time-effectiveness. As often overlooked, the benefit of robotics inclusion in many technologies, however, brings automation and efficiency that offer increased productivity and reduced costs and thus help in meeting sustainability goals. Bioprinting supported with robotics could be economically viable, safe, and healthful for workers, communities, and consumers.

Social sustainability

3D bioprinting has to go too far to be explored for its full potential for human organ printing and transplant; however, it holds a variety of mid-term and short-term benefits with positive ethical and social consequences. These consequences primarily include organ-on-chip as an alternative to animal testing, personalized medicine, tissue engineering, and disease modeling. The innovative promise to print human organs is the most striking solution to organ replacement avoiding sociocultural issues relating to possible gaps of inter- and intra-species integrity. However, the lacuna between promise and reality is big and one cannot overlook the dependency on high socio-ethical solutions of the society. The technology is in infancy and still offers expensive technological solutions that are likely to primarily benefit research-oriented works instead of real consumers. Furthermore, in case of bioprinted implants, patients do not readily accept implants due to the fear of developing cancer, dislodgement, and migration of implant. Although many studies showed short-term success, more long-term in vivo studies are required to be conducted to know if any side effect emerges in due course. Furthermore, despite the promises of personalized medicine, the unfavorable or adverse drug reactions are tested by standard “organ-on-a-chip” devices. In such cases how can a medicine or formulation be so personalized?

It is pertinent to discuss that sharing of personalized data or any type of digital content related to bioprinting of customized products has opened the debate on Intellectual Property (IP). Bioethical and legal issues of bioprinting and its various applications in medical and research domains have not yet been widely discussed among global scientific and bioethicists fraternity. The scope of bioprinting covers not only the issues of the advanced technologies of human tissues and organs printing but also raises a spectrum of interdisciplinary issues related to modern science, technology, bioethics, and philosophy. The process of printing a customized product especially for biomedical purposes is a complex nexus of series of activities or methods developed by expertise of different domains. A cumulative effort from biochemists, medical personals, researchers, and engineers enables to turn an idea into a customized product with anticipated qualities. At this juncture, the demand for the ownership of results may create legal and monetary consequences and conflicts among the contributors. Therefore, to bridge this gap, strong legal protections, development of regulatory agencies, and policies are needed under which the use of open access data repositories, production and promotion, and even monetization of a bioprinted product could be accomplished.

Conclusion

3D printing applications in biotechnology is expanding day by day in various scientific domains with the increasing focus on sustainable manufacturing. A comprehensive understanding of printing procedure and rational use of raw materials can help promote bioprinting as a sustainable technology. In the field of tissue engineering, sustainability is the biggest challenge that can be addressed through the development of green biomaterials with zero toxicity, for example, pectin-based biomaterial to fabricate patient-specific scaffolds (Lapomarda et al. 2020). 3D bioprinting is creating new products, new applications, new product verticals, and new market. Sustainability of this technology is the key for its future growth. This technology is both reducing existing jobs and creating new employments. However, there is a gap in the supply and demand of skilled manpower in this technology sector. This technology in the beginning has the same disadvantage like any new technology in penetrating the large market segment. The improvement in technology shall be linked to its larger acceptability in society and in turn shall bring more sustainability to it in future.

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3D printing in biotechnology: policy intervention

10

Chapter outline

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The trending developments at global level demand focus on creativity, novelty, and innovation in almost all spheres of life. 3D printing is an exponential technology that is accelerating industry 4.0 (Fraske, 2022; Popov et al., 2022). In the growth of biotechnology in research as well as industries, particularly in the areas of health-care, agriculture, waste management, bioenergy, and food and medical devices, 3D printing technology has shown immense potential in transforming and reshaping the R&D process (Crook, 2020).

Although the improvements in education, hygiene, socioeconomic status, lifestyle, regular exercise including yoga, and advances in engineering and medical sciences have led to an increase in the quality of life and lifespan in India (Ahmad et al., 2022), however, it is also a fact that there has been huge shortages of medicines, medical devices, diagnostic kits, PPE kits, ventilators, and food materials during the second wave of COVID-19 at a global scale. At one point of time, the entire supply chain and the healthcare infrastructure collapsed, irrespective of developed or developing countries (Ref). COVID-19 cases still exist with new variants and we are now adapting to live with it. At this juncture, 3D bioprinting technology plays a crucial role not only in manufacturing the items for healthcare purposes but also holds a tremendous promise in providing food security and sustainability in lockdown hit areas (Ref). Moreover, in times when the entire world population needs to keep itself away from the COVID-19 by implementing precautions such as wearing masks, maintaining social distancing, and frequent sanitizing hands and stuff to be used and having a prolonged wait for a miracle drug or vaccine, 3D printing with its applications in biotechnology has shown a ray of hope through recent developments in edible-printed vaccines and medicines that have desired release kinetics (Anwar-Fadzil et al., 2022).

Moreover, the impression of 3D bioprinting in biotechnology is dynamic and vital and is uplifting the medical and clinical research to new statures through exploring unknown and still untouched avenues. Not only in tissue engineering and

regenerative medicine, organ transplantation, drug discovery, and drug printing, but 3D bioprinting has also opened new vistas in clinical research including cancer, central and peripheral nervous system diseases, cardiovascular, liver and kidney research with the help of bioprinted tissues, organoids, and model organs. A detailed description of the applications of 3D bioprinting in medical and clinical research is recently reviewed by [Bejoy et al. \(2021\)](#). The advancements and applications of 3D bioprinting in medicinal and clinical research are primarily governed by the need for fulfilling the demand for age-related ailments in aging populations, minimizing the animal use in drug testing and clinical research and “less in availability but high in demand” organ donors.

Thus bioprinting reflects the simultaneous progression of sociotechnical imaginaries with bio-innovation resulting in more viable and lucrative future of using live tissues, organoids, and organs for regenerative and bespoke medicine, realistic miniature models for drug screening and testing, and even tailored personalized food ([Jin et al., 2021](#); [Nielsen, Kaldor, Irwin, Stewart, & Nicol, 2021](#); [Lafontaine, Wolfe, Gagné, & Abergel, 2021](#)). However, on the other side of this temporal landscape of the uniqueness and scope of potential applications of 3D bioprinting in biotechnology, the technology faces broad ethical issues ([Chadha et al., 2022](#)). Such issues are primarily raised due to the inclusion of living cells from varied sources and the use of bioprinted products either in elementary research (investigation of developmental and/or behavioral processes of living organisms) or in pharmaceutical and therapeutic applications (diagnostics, drug screening or testing, or implantation of bioprinted products). Thus all three stages of bioprinting, that is, pre-bioprinting, bioprinting, and post-bioprinting require certain level of ethical consideration and policy intervention to develop regulatory frameworks to address these issues ([Bejoy et al., 2021](#)).

Nevertheless, given that eventual aim of 3D bioprinting is to improve human health and upgrade the lifestyle without compromising planetary health that too in lack of proper socio-ethical concerns regarding possible consequences of bioprinting, the chapter outlines certain preliminary ethical and regulatory issues that are in need to be considered while using 3D bioprinting technology both at laboratory as well as commercial scale ([Shapira & Dvir, 2021](#); [Mukherjee, Sinha, Maibam, Bisht, & Paul, 2022](#)). Such discussions may relate to supervising and dealing general public expectations and the persistent reliance on technology-based scientific innovations ([Vermeulen, Haddow, Seymour, Faulkner-Jones, & Shu, 2017](#)).

Tissue engineering and regenerative medicine: ethics and policy

Regenerative medicine and tissue engineering is one of the rapidly emerging interdisciplinary fields in the area of biomedical research that deals with healing, replacement, and/or structural-functional improvement of diseased and/or injured tissues. 3D bioprinting has shown extensive applications in this field through cell

culturing, their incubation into biocompatible 3D-printed scaffolds, and developing or maturing these cells into tissues with desired functionalities. In comparison to the conventional tissue engineering techniques, bioprinting has become more relevant in understanding the geospatial relationship of cells among all the desired tissue components.

However, as the viable cells are the most important components of tissue engineering, special attention needs to be given to the source and further use of cells that hold particular ethical and regulatory issues (Nur Syamimi, Muhammad Aa'zamuddin, Azran, Munirah, & Abdurezak, 2022).

As far as the source is concerned there are: (1) Human and non-human embryonic stem cells; (2) Human and non-human pluripotent stem cells; (3) Autologous stem cells; and (4) Others such as plant and microbial cells.

Early human embryo-derived embryonic stem cells are the most common sources of live cells utilized for bioprinting; however, this remains debatable on the grounds of humanity and human dignity. The use of embryos to derive stem cells or any other research is highly regulated in many jurisdictions. Several specific laws and acts have been proposed against this, for example, the research involving Human Embryos Act 2002 (Australia) and Human Fertilisation and Embryology Act 2008 (United Kingdom). Co-concern has also been given to consent or exploitation of women for obtaining human embryos for research purposes. Jurisdiction in countries like the United States and India allows women to either donate or sell their fetus. Countries like Australia and Canada have strict laws for research involving human embryos. These jurisdictions allow couples or women to donate embryos that were developed through assisted reproductive technologies but are no longer obligatory for pregnancy.

In comparison to human embryonic stem cell, the pluripotent stem cells, by being not so controversial, are frequently used as their alternatives in 3D bioprinting. However, as the cell lines derived from adult cells often lead to cancers, the use of pluripotent stem cells strictly require the attention toward issues of safety and quality assurance prior to donation and procurement. Moreover, besides elementary studies of cellular behavior, disease emergence or drug testing is the major aim of implantation of bioprinted objects in a patient as a therapy; major concerns include the stability and biocompatibility of printed object against human health and safety. In case of autologous stem cells, although their use may avoid critical rules and regulations, the risk of forming teratomas exists, which can challenge the issue of health safety.

In summary, whatever source of cells is to be used in bioprinting of objects for medical and therapeutic purposes, there should be strict policies for protecting the interests of the donor and for obtaining and securing legitimate consent, confidentiality, and relinquishments of ownership rights. Such steps may lead to minimal risk of harm and potential violation of interests (Matheu, Busby, & Borglin, 2018; Singh et al., 2022).

On the contrary, the regulatory concerns for obtaining non-human embryonic and adult stem cell lines or cells derived from other sources, such as plants and bacteria, virus, etc., is much less stringent than human-derived cells; however, due to

potential risk of developing immunological problems that xenogeneic cells may cause and zoonosis, that is, transfer of infectious disease from animals to humans, here the animal welfare, health, and safety from biohazards are the primary ethical concerns (Skya, 2022). In such cases, the laws or regulations should focus on reducing the risk of transmission of disease across species and potential creation of chimeras. Thus in addition to proper rules, researchers and individuals working in this area should be aware of biosafety plans and regulations on biosafety hazards (Mota, Maciel Braga, Cabral, & Filho, 2022).

Organoid research: ethics and regulatory aspects

One of the major uses of stem cells include the development of organ-like structures called organoids, which are now being used in the toxicology research, investigation of disease occurrence, and drug development. The recent couple of years witnessed a rapid upsurge in organoid research. Variety of organoids is being developed to mimic human organs, particularly brain, liver, pancreas, kidney, and some endocrine glands such as thyroid. Even tumor organoids, also known as “tumoroids”, have been developed to investigate the etiology and cellular behavior of cancerous cells and also to assess novel therapeutic strategies. These 3D-bioprinted structures offer similarities in terms of three-dimensional cellular architecture, cellular diversity of the corresponding tissue, and the functionalities of the original organ *in vivo*.

As organoids are developed from cells and tissues from human individuals a critical ethical discussion is needed. Also, the practice of personalized drug testing using organoids developed from patient-derived cells may perturb the usual line of preclinical and clinical trials of drug screening and development. However, the major aspect in using organoids for research and/or clinical purposes is establishing globally recognized regulatory procedures for donor consent, informing clinically relevant findings to the patients and long- or short-term storage of organoid in so-called bio-banks.

Furthermore, collaborations between the research and commercial front may require vast data exchange that may eventually bring new concerns around permissions, privacy rights, ownerships, intellectual property rights, safety issues, confidentiality decorum, commercialization, and marketing (Kolade, Adegbile, & Sarpong, 2022). Furthermore, during international collaborations, due to patenting concerns, global patenting rules for organoid rights should be considered. The production of big data, inclusion of multi-omics, bio-banking, and the globalization of the different subdomains of biotechnology (microbial, medicinal, environmental, etc.) has complicated the task of setting universal ethical standards.

Nevertheless, the regulatory guidelines for organoid research and its use in transplantation purposes are not very well laid and urgently require scientific and logical attention and an update. The technical, ethical, and regulatory issues in association with organoid development and their utilization irrespective of research or clinical

purposes are subject to all-encompassing regulatory scrutiny and controlled by government considering social, cultural, and economic and other relevant facets of corresponding country (Sekar et al., 2021). Moreover, there should be certain common rules and regulations that are formed and followed as international laws equally by different countries in case of foreign collaborations. Specific committees should be formed that assess the current state of art in organoid research, judicially investigate the benefits and hazards, address issues associated with ethical problems, including donor consent, long- or short-term storage of organoids, and evaluate appropriate supervision strategies as per the government guidelines (Rinaldi & Colotti, 2019). While several European countries have individual regulatory organizations, in India, the Institutional Committee for Stem Cell Research (ICSCR) extends its role as a regulatory agency for all inclusive stem cell research. At the commercial front, the manufacturing and development process of organoids must follow similar customary criteria of good manufacturing practices as any pharmaceutical drug. Furthermore, both at research and commercial fronts, regulations and laws related to the heterogeneity of permitted quality standards, privacy, data protection, patenting, and ownership rights should be followed. Keeping these requirements in mind, recently, updated stem cell research guidelines are proposed by the ISSCR. It is expected that organoid-based biotechnology innovations would need updated global regulatory guidelines and governance in the future (Mukherjee et al., 2022). However, ethical, moral, and legal existential questions still persist. Last, but may not be least, strong public curiosity in organoids coupled with nascent and immature nature of organoid research area needs judicious attention aiming at communication between scientific personals, general public, and industry to avoid inaccurate representations and over expectations (Mota et al., 2022).

Ownership of bioprinted product

Giving that one of the major aims of bioprinted objects is to use them as implants inside a human body, 3D bioprinting opens a new window different from previous legal regulations on medicines, life-saving transfusion stuffs such as blood or plasma, wherein two parties are involved as donor and recipient, surgical items, or on conventional 3D printing. In case of bioprinting and bioprinted objects the prime concern basically is the issues associated with ownership and the possible benefits of bioprinted products to different interested parties that may include medical personals, researchers, donors, recipients, and product manufacturing biotechnology industries. In addition, there is also an essential need for maintaining an individual's (patient or recipient) autonomy over their bodies. A multiparty regulation between the medical and legal professions is required to avoid new systems of exploitation. For example, black marketing of biofabricated organs or exploitation of donors. In addition to the donor and recipient, as the process of bioprinting of an implantable object is a complex nexus of series of activities and/or methods developed by expertise of interdisciplinary domains, such as scientists, medical

professionals, engineers (designing and operating the bioprinter), and computer professionals, the demand for ownership may create legal and monetary consequences and conflicts among the contributors. Therefore, to bridge this gap, strong legal protections and policies are needed under which the right of each contributor, the use of open access data repositories, production and promotion and even monetization of a 3D-printed object could be preserved. Furthermore, the issue of donor consent restricts only for obtaining cells as initiating material and not for the procedure of organ biofabrication. Moreover, the bioprinted transplantable human organs are developed artificially under controlled and distinguished conditions outside the human body, thus, exempting the risk of health or life of cell donor. With these unique properties, the 3D-bioprinted transplantable organs, prior to their transplantation in a human body, might be treated as the objects of civil rights. However, post implantation of organs and tissues in a body, the bioprinted object must be accepted as an integral part of the body and with the death of recipient the legal death of this organ will be denoted. Thus, it is important to form certain strict rules and policy to recognize these facts and also for the subsequent termination of ownership along with the parallel prohibitions and limitations on their removal and subsequent sale, associated with obtainment, sale, or purchase human biomaterial (Mufarrih et al., 2021).

Bioprinting: effect on supply chain

The current supply chain model is based on globalization. Long travel shipments and complex supply chain put additional cost to product. There are many factors that impact or disrupt the supply chain. The recent example of supply chain disruption has been the lockdown during the ongoing COVID-19 pandemic throughout the world in phased wise manner. 3D printing technology is proving a boon to shorten the supply chain. Russia-Ukrainian crisis may lead to reverse globalization to some extent, causing further challenges to supply chain. To overcome these challenges, there is need for manufacturers to produce items closer to end user at low cost. In this scenario, 3D printing technology has potential to meet some of the future challenges of society arising out of prevailing and emerging situations.

Although bioprinting technology and availability of bioprinted products affects the supply chain through local manufacturing and utilizing the material available in proximity; however, the disruption of supply chain and its inadvertent impact on associated communities should be considered.

The on-demand localized 3D-printed objects for clinical purposes may disrupt different components of traditional goods supply chain, such as production, post production processing, and marketing. With increase in market size of portable and easy-to-handle bioprinters, the technique will allow users to bypass the manufacturer and print patented as well as copyrighted designs (Tong et al., 2021). If 3D bioprinting becomes more accessible, it could create different types

of supply chains of medical goods and devices leading to theft of intellectual property, and loss of control of the traditional manufacturer over that property. In addition to creating such issues, it may also negatively influence the workforce requirements in different phases of traditional production and supply. The localized biofabrication may also create challenges like how such bioprinted objects will be monetized.

IPR and patenting landscape in bioprinting

While working in the area of bioprinting there must be an awareness of the applicable Intellectual Property (IP) framework and the need to protect their interests prior to both cases, that is, publishing and patenting of research results and products, respectively, or transfer to clinical trials (Bicudo, Faulkner, & Li, 2021a,b,c). Important subareas of focus include bioprinters, methodology, materials, computational data, bioprinting processes, and bioprinted products such as organoids, 3D scaffolds, or transplantable human organs. However, due to the complex combination of interdisciplinary domains of engineering and biophysics (designing of bioprinter and optimizing the physical parameters of process), computer science (optimizing the CAD/CAM component), material science (selection of non-toxic and biocompatible natural or synthetic polymers for hydrogels), and medicinal biochemistry and biotechnology (drug development and therapeutics), the bioprinting technology possesses certain level of uncertainty within the intellectual property legal landscape. This uncertainty appears over what aspects or whose contribution should be protected and what aspects should be considered as patentable and subjected to copyright for ethical or public policy reasons.

Bioprinting starts in two ways, wherein the first way is a 3D scan of a real-life object and the second way includes the creation of a digital representation of such an object through computer-aided design software or CAD software. CAD files and CAD software are copyrightable and that copyright could even be used to protect from reproducing the images in the file (Bicudo et al., 2021a,b,c). In India, copyright act also considers the novelty and originality of the work, wherein originality is defined by those pieces of works that are somehow different in properties, contain certain degree of intellectual effort and creativity. Thus keeping this standard in mind, a CAD file that is designed by the software could be copyrightable but only a scan or picture of a work may not be sufficiently original and copyrightable. As far as patenting is concern, patents could apply to new or novel methodologies evolved for 3D bioprinting, bioprinter designing, and post-bioprinting maturation processes. However, because of the customized and bespoke nature of bioprinted objects, their patenting may not be very valuable and there exists an ambiguity as in many jurisdictions patenting of human organs or tissues is not allowed while in some, printed products derived from the materials isolated from the human body or otherwise fabricated through any already documented or novel technical process can be observed as patentable (Althabhwawi & Zainol, 2022; Nielsen et al.,

2021; Birtchnell, Daly, & Heemsbergen, 2020). With reference to these directives, 3D-bioprinted tissues using hydrogel and a scaffold may be patentable but a 3D-printed organ produced by 3D scanning and computer may not.

Conclusion

3D bioprinting technology has made it possible to 3D print the human stem cells, plant cells and microbial cells, and even food. The technology is the future of agriculture, food, medicine, and medical devices. Emerging engineering approaches are transforming the entire health sector from medicine to medical devices to personalized healthcare. It has made possible the concept of idea to market. There is a need for developing novel biomaterials, which can be used for a variety of purposes, such as nanomaterials for nanomedicine and drug delivery; tissue engineering for developing miniature tissue models; biosensing wearable devices that can continuously monitor various health parameters and can be connected with mobile using Internet of Things; scaffolds for bone regeneration; hydrogels for dental implants; medical devices which can be used in sustaining the functioning of vital body organs that get affected in diseased conditions. There is now a growing global demand for personalized prosthetic implants and diagnostic tools for early detection of the disease, its progression, as well as its management. Tremendous advances in molecular biology and understanding of disease processes have been made globally and based on that it is possible to develop pharmaceuticals that can help in providing better healthcare. 3D printing of drug is an emerging area. Nowadays, drug resistance is a major challenge. Transition from synthetic drug to a biological drug is a major trend in global pharmaceutical education, research, and innovation. There is a need to integrate reverse pharmacology with modern drug discovery approaches, such as bioinformatics, to fuel investigational drug pipelines. Genetic and lifestyle data of patient can be useful in prevention, diagnosis, and treatment of diseases and developing precision medicine. Microbiome engineering is another area that helps understand the mechanism behind various diseases through host-microbe interactions and gut-organ axis (such as gut-liver axis, gut-lung axis, etc.) studies to help develop precision medicine and precision probiotics. 3D printing technology helps fabricate these tissues and organs to support such studies.

New diagnostics, which are used as a point of care (PoC) have been developed using microfluidics chip technologies. Lab-on-chip (LoC) devices based on the integration of photonics and microfluidics are used for miniaturizing laboratory workflows in single-cell omics based on digital and droplet microfluidics, drug discovery and development, and clinical diagnosis. Microfluidic organ-on-chip (OoC) devices are used in the development of miniature 3D tissue models, such as organoids-on-chip and disease-on-chip, for drug screening. 3D bioprinting methods such as inkjet, extrusion-based, digital light processing (DLP), microfluidics, and two photon polymerizations are used for developing living tissues and organs, bone regeneration, and blood vessels. Today, 3D-printed mask and skin tissues are

transforming the cosmetics industry. There is need to integrate robotics with 3D printing for wider applications in the health sector. Due to differences in the physiology of human and animal systems, after successful efficacy and preclinical animal studies, many drugs fail in clinical trials during human settings. The organs/organoid developed using stem cells through 3D bioprinting or microfluidic OoC devices can accurately model the structure and function of human organs to provide better success in drug screening and thus the development of drug. Thus these novel technologies will reduce our dependency on animals for clinical trials of drug and in turn protect our ecosystem. Microfluidic devices also have applications in synthetic biology, RNA technologies, CRISPR-based gene and genome editing, and cell and gene-based therapies. There is need to design novel photonic, optofluidic, optoelectronic, microfluidic, and nanofluidic devices that can be produced with a wide variety of materials through digital and precision manufacturing and further can be integrated with artificial intelligence and IoT approaches for wider applications in healthcare ([Christou & Tsoulfas, 2022](#)).

For the growth and development of 3D bioprinting technology, there is a need to bring together researchers, scientists, academicians, and engineers from pharmacy, bioscience, biomedical science, biotechnology, microbiome engineering, multi-omics, bioinformatics, biomedical engineering, bioengineering, tissue engineering, nanotechnology, computer engineering (AI, ML, AR/VR, blockchain, data science, software engineering, cyber security, and bigdata analytics), electrical, electronics and instrumentation engineering (bioMEMS, IoT, optics and photonics), mechatronics, robotics, medical technology, material and fluidic technology (microfluidics and μ TAS), cyber physical system, telemedicine, digital twin technology, additive and subtractive manufacturing, digital and precision manufacturing, technology management, quality control, and quality assurance as well as managers, technologists, clinicians, public policy experts, entrepreneurs, and industries with a goal to develop capability for novel drug and vaccine and rapid scaling up of medical device fabrication under certified conditions and those technologies can be transferred to industry for large-scale manufacturing, so as to quickly introduce new technologies in the healthcare sector. Furthermore, there is a need to produce skilled manpower with global competitiveness to meet the demand of 3D bioprinting industry.

With these advances, 3D bioprinting technology will be able to explore real potential for understanding, diagnosing, as well as developing new therapies. The major aim of 3D-printed cells and living structures is their use in medical research, in vitro drug testing, and as in vivo transplant materials, which raises ethical, policy, and regulatory challenges at all three stages of the bioprinting process in relation to the objective of research and its further applications ([Horst & McDonald, 2020](#)). The present chapter emphasizes the ethical and regulatory issues arising with 3D bioprinting processes and identifies ethical issues in relation to the use of humans and animals as a source of materials and cells; the quality and safety of cell and materials; organoid research, ownership of bioprinted products, and supply chain disruption. In addition, it also explores the regulatory issues related to the copyrights of 3D-bioprinted materials and products, as well as intellectual property and patenting rights.

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3D PRINTING IN BIOTECHNOLOGY

Current Technologies and Applications

3D Printing in Biotechnology: Current Technologies and Applications explains the basic designs and recent progress in the application of 3D printing within various biotechnology fields. It is a compilation of the fundamentals, designs, current applications, and future aspects related to this emerging technology and summarizes the promising application of 3D bioprinting. The chapters contain detailed state-of-the-art knowledge to assist in the development and design of 3D printing with applications in the medical, food, and environmental science fields. This book will appeal to researchers as well as students from different disciplines, including materials science, food, agriculture, and various biomedical fields. The content includes industrial applications and fills the gap between the research works conducted in the laboratory to practical applications in related industries.

Key features:

- Introduces the emerging technologies and sectors in the field of 3D printing
- Discusses the development of sustainable materials and bioinks and provides a guide for medical professionals and practitioners to incorporate current 3D printing technology into their medical practice
- Bridges the knowledge gap for current designs used in 3D printing technology for designing an efficient and innovative 3D printer
- Previews the technological basis for new farming practices and food engineering concepts utilizing 3D printing techniques

About the authors:

Dr. Nandita Dasgupta has vast working experience in nanomaterials and toxicology and is currently working at International Research Center in Critical Raw Materials for Advanced Industrial Technologies (ICCRAM), Universidad de Burgos, Burgos, Spain.

Dr. Vineeta Singh is currently working as an assistant professor at the Institute of Engineering and Technology, Lucknow, India, and has 15 years of teaching and research experience.

Dr. Shivendu Ranjan has extensive expertise in micro/nanotechnology and biotechnology and is currently working as an assistant professor, School of Nano Science and Technology (SNST), Indian Institute of Technology, Kharagpur, West Bengal, India.

Ms. Taijshee Mishra is currently working as a consultant, NITI Aayog, Government of India.

Dr. Bhartendu Nath Mishra is Dean, Research and Development and Professor of Biotechnology at Dr. A.P.J. Abdul Kalam Technical University, in Lucknow, Uttar Pradesh, India.



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